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Original Article

# Observation of variations of biochemical and mineral contents in bone tissue using x-ray diffraction technique

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***Abstract***

*The concentrations of calcium (Ca), magnesium (Mg), and phosphorus (P) in aluminum-exposed bone tissues were found to be significantly lower compared to the control group. However, treatment with chelating agents DFO and DFP led to a notable increase in these mineral contents. Trace element concentrations were analyzed using ICP–OES. This study suggests that aluminum toxicity results in considerable depletion of bone minerals and a reduction in key biochemical constituents. Bone tissue is a dynamic composite biomaterial composed of organic matrix and inorganic minerals, primarily hydroxyapatite. Variations in its biochemical and mineral composition can reflect physiological and pathological changes. This study investigates the mineralogical and structural variations in bone tissues using the X-ray Diffraction (XRD) technique. Bone samples were collected and analyzed for their phase composition, crystallinity, and mineral content distribution. The XRD analysis revealed significant differences in peak intensities and crystallite sizes, indicating alterations in mineralization patterns. These findings offer valuable insights into bone health assessment, disease diagnostics, and the effects of external factors such as age, diet, and environmental exposure on bone quality.*

***Keywords***: *Bone tissue, X-ray diffraction, hydroxyapatite, mineral content, crystallinity, biochemical variation, structural analysis*

## Introduction

Aluminum (Al), the third most abundant element in the environment, exists primarily as a trivalent cation and is widely found in plant and animal tissues as well as natural water sources [1,2]. Human exposure to aluminum can occur through diet, drinking water, environmental sources, and the consumption of aluminum-containing medications. Primary exposure routes include ingestion and inhalation of aluminum particles. It is believed that aluminum disrupts calcium metabolism, increasing the risk of osteoporosis by impairing calcium absorption and bone mineralization. Bone, a complex hierarchical and heterogeneous structure, exhibits excellent biomechanical properties due to its combination of organic (collagen) and inorganic (calcium phosphate) constituents [3,4]. Studies have indicated that aluminum accumulates in bones and soft tissues such as the liver. Consequently, this study investigates how aluminum toxicity affects biochemical constituents and mineral composition in bone tissue [5,6].

## Materials and Methods

### Animals

Male Swiss albino mice (25±2 g) were obtained from the Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College, Annamalai University. They were housed at 25 ± 1ºC with a 12-hour light/dark cycle and provided



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with food and water ad libitum. Ethical clearance was granted by the Institutional Animal Ethics Committee (Reg No. 160/1999/CPCSEA, Proposal No. 851).

1. **Treatment Schedule**

   Mice were divided into four groups (6 mice each):

   Group I: Control (standard diet and water).
   Group II: Aluminum intoxicated (100 mg/kg b.wt./day).
   Group III: Aluminum + DFP (0.72 mmol/kg).
   Group IV: Aluminum + DFP + DFO (0.89 mmol/kg).

   Treatments lasted for 16 weeks. Aluminum was administered orally as aluminum chloride. Group III received DFP orally, while Group IV received DFP and DFO intraperitoneally. Twenty-four hours after the final chelation dose, mice were sacrificed, and bones were extracted and stored at −80°C.

2. **Test Chemicals**

   Deferoxamine (Desferal) and Deferiprone (DFP) were procured from Novartis and Sigma Aldrich, Mumbai. The purity levels were 99.9% and 98% respectively. Structures were drawn using ChemDraw Ultra 7.0.1.

3. **Bone Sample Preparation**

   Bone samples (n=6 per group) were pooled, lyophilized, powdered using ball milling, and stored in a desiccator. X-ray diffraction analysis was performed using an X'PERT PRO–PANalytical Diffractometer (CuK$\alpha$ radiation) at CECRI, Alagappa University.

**Results and Discussion**

This study evaluated the protective effects of DFO and DFP in aluminum-exposed bone tissue. XRD analysis revealed loss of minerals in aluminum-treated groups and recovery after chelation. Characteristic peaks of MCP, CP, OCP, CC, and CMP were identified [7,8] as shown in table 1 and figure 1.

**Table 1. For Comparison of intensity of minerals content in the bone tissue of Mice (mus musculus) at wavelength (λ) =1.54060 Å of Rhomb-centered lattice Aluminum.**

| Control | | | Aluminum intoxicated | | | Al+DFP | | | Al+DFO+DFP | | | Minerals |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2θ | D | I | 2θ | D | I | 2θ | D | I | 2θ | D | I | |
| 27.020 | 3.297 | 46.600 | --- | --- | --- | --- | --- | --- | 27.025 | 3.291 | 47.345 | MCP |
| 31.684 | 2.822 | 100 | --- | --- | --- | 31.432 | 2.785 | 69.289 | 31.724 | 2.955 | 98.273 | CP |
| 39.030 | 2.306 | 10.420 | 38.972 | 2.301 | 9.476 | 38.970 | 2.302 | 10.291 | 39.067 | 2.304 | 9.456 | OCP |
| 43.980 | 2.057 | 15.390 | --- | --- | --- | --- | --- | --- | 43.924 | 2.026 | 21.745 | CC |
| 45.430 | 1.995 | 28.360 | 45 | 1.981 | 7.865 | 45.021 | 1.982 | 18.857 | 45.519 | 1.992 | 30.981 | CMP |
| 56.420 | 1.629 | 8.110 | --- | --- | --- | --- | --- | --- | --- | --- | --- | CP |

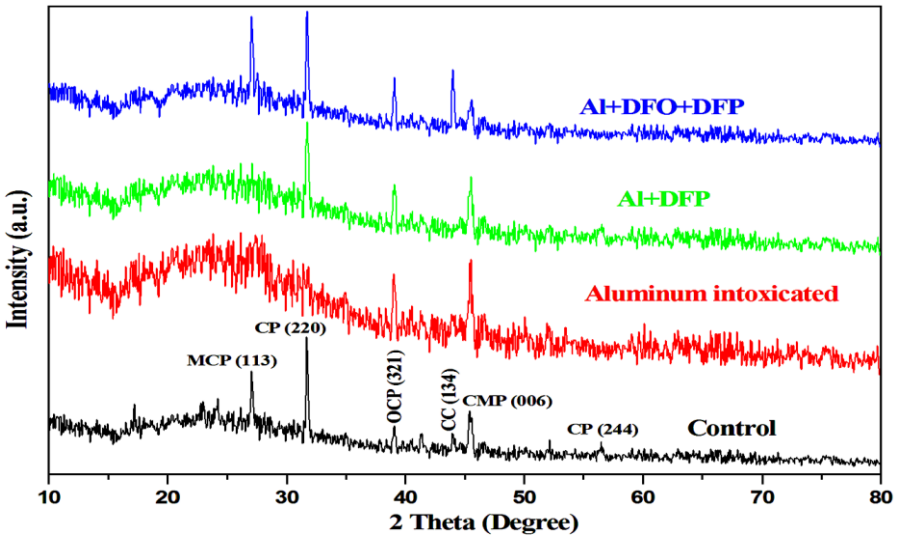


**Figure: X-RD characterization**

The XRD spectra indicated that aluminum toxicity reduced bone crystallinity and mineral content, which was restored by chelation therapy.

**Conclusion**

XRD analysis confirmed that aluminum exposure results in significant loss of bone minerals. DFP alone showed positive effects, but a combination therapy of DFO and DFP proved more effective in restoring mineral levels and biochemical balance in bone tissue.

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