Analysis of morphology and absorption of calcium and magnesium for calcium phosphate \(\text{Ca}_3(\text{PO}_4)_2\) in rat’s spine

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Abstract. The decline in bone mass is a sign of metabolic diseases called osteoporosis. Osteoporosis can be treated by adding the calcium level in the form of nano sized \(\text{Ca}_3(\text{PO}_4)_2\). One type of calcium which can build and strengthen the bones is \(\text{Ca}_3(\text{PO}_4)_2\). This study aimed to determine the effectiveness of nano sized \(\text{Ca}_3(\text{PO}_4)_2\). addition which can be absorbed by rat bones and to identify the crystalline structure that was formed in rat bones with a specific purified diet. Nano sized \(\text{Ca}_3(\text{PO}_4)_2\) was varied in a composition 0.5X, 1.0X and 1.5X compared to the normal needs in the purified diet. The rats were harvested in every two weeks, then their spines were taken to be investigated. The rat’s spines were examined by using X-Ray Diffraction (XRD) and Atomic Absorption Spectroscopy (AAS). The effective amount of calcium for the rats was at the value of 0.5X. The amount of Ca as a bone mineral increased as the increasing amount of nano sized \(\text{Ca}_3(\text{PO}_4)_2\), while the amount of Mg tended to fluctuate. The crystalline structure of hexagonal type was found in the rat bones with. Lattice parameters of \(a = 9.50\) Å and \(c = 6.83\) Å.

Keywords: \(\text{Ca}_3(\text{PO}_4)_2\), purified diet, atomic absorption spectroscopy, X-Ray Diffraction, hydroxyapatite.

1. Introduction

Osteoporosis is a disease that affects bone in elderly people, which the initial symptom is not visible whether it can be perceived. International Osteoporosis Foundation (IFO) said that one of four women in Indonesia with an age range of 50–80 years was infected by osteoporosis. According to WHO, in 2050 the number of osteoporosis sufferers may be increased three times. The risk of being an osteoporosis sufferer in Indonesian women is four times higher than men, it is due to osteoporosis that may afflicts the women in menopause period. The loss of oestrogen after menopause increases the risk of osteoporosis. There are several causes of osteoporosis, such as unhealthy lifestyle, lacking exercise, eating junk food, smoking and drinking alcoholic beverages.

Researches on osteoporosis treatment have been conducted. For example Rollo et al. [1] analyzed microarstektur the trabecullar on the osteoporosis. Several similar studies have been made, such as research conducted by Darmadi Ts [2] and Elkomy and Elsaid [3] about the treatment of osteoporosis using a tungak and herbal plants. Other research had been conducted by Aulyani et al. [4] which is about absorption \(\text{Ca}_3(\text{PO}_4)_2\) in rats with unapplied ovariectomy.

Research on the treatment of osteoporosis and the absorption of calcium on bone has been conducted. From the previous studies, research on the absorption of calcium phosphate in ovariectomized rats has not been done. Therefore, this study aims to observe the absorption capacity and the size of the crystalline by the addition of nano- \(\text{Ca}_3(\text{PO}_4)_2\).due to osteoporosis bones.
Table 1. Mineral composition of rat’s bone sample by AAS (in ppm)

| Sample Code | Variation               | Time     | Ca ppm | Mg ppm |
|-------------|-------------------------|----------|--------|--------|
| TO          | Non ovariectomized      |          | 207    | 68.56  |
| O2M         | Control                 | Ovariectomized (2 weeks) | 192 | 54.8   |
| O4M         | Ovariectomized (4 weeks)|          | 168.5  | 59.98  |
| 2M0.5X      | 2 weeks                 |          | 5390   | 66.8   |
| 4M0.5X      | 0.5                     | 4 weeks  | 16070  | 254.5  |
| 6M0.5X      | 6 weeks                 |          | 16220  | 224    |
| 2M1.0X      | 2 weeks                 |          | 6108   | 65.4   |
| 4M1.0X      | 1.0                     | 4 weeks  | 16345  | 207    |
| 6M1.0X      | 6 weeks                 |          | 16770  | 222.5  |
| 2M1.5X      | 2 weeks                 |          | 9140   | 106.5  |
| 4M1.5X      | 1.5                     | 4 weeks  | 17500  | 173.5  |
| 6M1.5X      | 6 weeks                 |          | 17955  | 206.5  |

2. Materials and methods

2.1. Preparation of sample
The sample were spines of *Rattus Norvegicus* rat with Wistar strain on the age of seven weeks. The rats fed with nano sized Ca\(_3\)(PO\(_4\)\(_2\)) in their purified diet. The compositions of the nano size Ca\(_3\)(PO\(_4\)\(_2\)) were 0.5X, 1.0X, and 1.5X of the normal needs. So that the samples were identified of four groups, namely rat control, rat with nano sized Ca\(_3\)(PO\(_4\)\(_2\))0.5X, rat with nano sized Ca\(_3\)(PO\(_4\)\(_2\))1.0X, and rat with nano sized Ca\(_3\)(PO\(_4\)\(_2\))1.5X. The rat control consisted of three distinct types of rats, such as non-ovariectomized rats, ovariectomized rats with the age of two weeks, and ovariectomized rats with the age of four weeks for all groups. Table 1 shows the value of Ca and Mg, which belongs to non-ovariectomized control sample and ovariectomized sample after two weeks and four weeks.

2.2. Characterization of sample
The samples were pulverized to facilitate the process characterization. Atomic Absorption Spectroscopy (AAS) and X-Ray Diffraction (XRD) were used to characterize the samples. XRD was performed by using Rigaku Miniflex 600 1 kV with CuK\(\alpha\) radiation and wavelength of 1.54 Å. The phases of the samples were determined by the Highscore Plus software to analyze the diffraction lines in a range of 5°–90°. The mineral concentration such as Calcium (Ca) and Magnesium (Mg) were examined by AAS.

3. Results and discussion
Table 1 presents the results of the calcium and magnesium compositions in the spin rat’s samples. The value of calcium (Ca) in the sample after being ovariectomized decreases than sample without ovariectomy. The composition of Ca increased because of the addition of varied nano sized Ca\(_3\)(PO\(_4\)\(_2\)) in feed might be well absorbed, so the bones contained high calcium level. However, the value of Ca after the treatment is quite high, so that the Ca level in blood is much as well. It is due to the content of Ca in the crystals are constant; less addition of Ca value can increase the absorption level of Ca immediately. High level of calcium that found in bone was the deposition of high calcium in blood plasma. Mg value is fluctuating caused by its role as inhibitor in bone that can detain repair for bone [5].

Figure 1 exhibit the diffraction pattern of bone samples with the variation of composition of feed and ages showing the counts versus angle 20 (°) From these diffractograms, the peak of (002) which refers to HAp can be observed. The size of Ca\(_3\)(PO\(_4\)\(_2\)) particle was in a range of ±75 nm and formed hexagonal crystalline structure of hydroxyapatite. The lattice parameters can be observed in table 2.
### Table 2. Lattice parameters of the rat’s spinal

| Sample Code | Variation       | Time        | a (Å) | c (Å) |
|-------------|-----------------|-------------|-------|-------|
| TO          | Non ovariectomized | 9.42      | 6.88  |
| O2M         | Control         | Ovariectomized (2 weeks) | 9.39  | 6.75  |
| O4M         | Ovariectomized (4 weeks) | 9.48  | 6.73  |
| 2M 0.5X     | 2 weeks         | 9.45      | 6.79  |
| 4M 0.5X     | 0.5X            | 4 weeks    | 9.57  | 6.83  |
| 6M 0.5X     | 6 weeks         | 9.45      | 6.80  |
| 2M 1.0X     | 2 weeks         | 9.59      | 6.85  |
| 4M 1.0X     | 1.0X            | 4 weeks    | 9.46  | 6.79  |
| 6M 1.0X     | 6 weeks         | 9.72      | 7.11  |
| 2M 1.5X     | 2 weeks         | 9.59      | 6.78  |
| 4M 1.5X     | 1.5X            | 4 weeks    | 9.42  | 6.78  |
| 6M 1.5X     | 6 weeks         | 9.45      | 6.81  |

### Figure 1. XRD patterns for the all samples

In addition to the presence of crystal structure, the amorphous phase can be found in the bone as well because the relatively small peaks were formed. So, this phenomenon denotes the presence of crystalline and amorphous phase. In ovariectomized sample, the amorphous phase decreased and the increasing value of Ca caused increasing value of amorphous phase as well until the amorphous level would become similar as non-ovariectomized. This phenomenon can be known, because the small...
Table 3. Crystalline size of the rat’s spinal

| Sample Code | Variation        | Time       | A (amorphous peak) | β (FWHM) | 2θ (°C) | D (Crystalline size) |
|-------------|------------------|------------|---------------------|----------|---------|----------------------|
| TO          | Non ovariectomized | 52         | 1.49                | 26       | 62.52   |
| O2M         | Control           | 48         | 2.25                | 26.6     | 43.92   |
| O4M         | Ovariectomized (4 weeks) | 45 | 2.38                | 25.5     | 31.74   |
| 2M0.5X      | 2 weeks           | 50         | 1.43                | 26.5     | 69.96   |
| 4M0.5X      | 0.5X              | 48         | 1.91                | 26.7     | 50.19   |
| 6M0.5X      | 6 weeks           | 52         | 1.64                | 26.3     | 67.11   |
| 2M1.0X      | 2 weeks           | 53         | 1.99                | 26.8     | 45.57   |
| 4M1.0X      | 1.0X              | 50         | 1.53                | 26.4     | 63.56   |
| 6M1.0X      | 6 weeks           | 51         | 1.33                | 26.1     | 74.21   |
| 2M1.5X      | 2 weeks           | 53         | 2.58                | 28       | 39.77   |
| 4M1.5X      | 1.5X              | 54         | 1.57                | 28.2     | 44.06   |
| 6M1.5X      | 6 weeks           | 48         | 1.55                | 28.4     | 53.98   |

peaks that are formed indicate the existence of crystalline and amorphous phase. The amorphous in the control has decreased because on the osteoporosis, crystals, which is experiencing a reduction in, and leaving huge crystal that led to the top of the amorphous. The value that determined crystalline size of bone can be observed in table 3.

4. Conclusions
The Ca value on the rat bones increased as the increasing amount of nano sized Ca\(_3\)(PO\(_4\))\(_2\) in purified diet. The content of Mg was fluctuating for the varied composition. The effective result of nano sized Ca\(_3\)(PO\(_4\))\(_2\) that was absorbed by the rats was shown by the purified diet composition at 0.5X. The bone samples before and after the treatment applied had the same crystal structure with the form of hexagonal, which referred to hydroxyapatite.

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