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Therapeutic Dose of *Madidihang* Fish Bone Flour and $CaCO_3$ towards Calcium and Phosphorus Content in Blood Serum and Bones of Ovariectomy Rat

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Abstract : Fish bone flour of *Madidihang* (*Thunnus albacares* Bonnattre) produced fine-shaped powder with yellowish white until yellow colour and has high content of Ca (calcium) and P (phosphorus). This research aims to determine the dose therapy of *Madidihang* fish bone flour deproteinization, non deproteinization and $CaCO_3$ towards high calcium and phosphorus in the blood and the bone of the ovariectomy rat. We used experimental methods with 27 female wistar rat (*Rattus novergicus*) with age 75 day in therapy use of *Madidihang* fish bone flour and $CaCO_3$ with doses of 0, 400, 800 and 1600 mg/kg bw day. Analysis of Ca and P parameters in blood serum and bone of rats is using AAS (automatic absorbance spectrophotometer). The results of in vivo test show that the highest value of Ca and P in blood and bone of rat is in the treatment of $CaCO_3$ for 8.273 mg/dL; followed by non-deproteinization 7.951 mg/dL and deproteinization 7.127 mg/dL. While the highest P found in deproteinization treatment of 5.407 mg/g dw and the lowest in $CaCO_3$ treatment for 2.014 mg/g dw. Otherwise, the highest Ca in bones of dose 1600 mg/g dw is deproteinization of 8.29 mg/g dw, and the lowest is $CaCO_3$ for 7.04 mg/g dw. As for the highest dose deproteinization P 1600 2,224 and low $CaCO_3$ 1,475 mg/g bk.
Keywords: Calcium and phosphorus, blood serum and bones, Therapeutic doses of *Madidihang* fish bone flour, ovariectomy rat.

Introduction

Ca (Calcium) is also an important element for the strength of bones and teeth found in many products, e.g. fish bone, milk, green vegetables, fruits and nuts. Fine resources of Ca are dairy products, but some people have intolerance to lactose or other personal reasons. Therefore, it is necessary to propose a natural source of Ca that is readily and cheaply available in nature to meet a fairly high Ca daily intake. Ca from bones and cartilage of marine fishes is available alternatives. However, to this date Ca bioavailability has not known clearly.

Bioavailability will affect the mineral in the body; therefore we need sources of Ca and P (phosphorus) which are easily found in nature but have a characteristic of high Ca and P solubility. High Ca and P not necessarily have the same level mineral bioavailability in the body. The term of nutrition ingredients of a substance to be considered as bio available is nutritional substances in the form of dissolved minerals (soluble)[1, 2]. However, not all types of dissolved minerals are bio available[3].

A source of mineral, especially Ca and P, is essential for metabolism of bone but Ca daily needs were commonly not met in a significant proportion. Ca oral derived supplement from the nature is not sufficient enough to be optimal. One alternative source of minerals that can help to meet the daily requirement with is high mineral resources in nature. The source of high minerals especially Ca and P derived from marine sources which are currently has not well known and utilised yet[4]. The high mineral content of bone fish flour is very suitable as a natural source of Ca, food products, animal feed or as a supplement[5]. One of flour bones of fish which are rich in minerals yet to be optimized from a source of maritime are waste bones of fish *Madidihang*

(*Thunnus albacares* Bonnattre). High bone density in Premenopausal can maintain Ca bone deposits thereby reducing the loss or decline of Ca during menopause. Thus, individuals with a high bone density during the growth to premenopausal period will be prevented from future postmenopausal osteoporosis[6]. Consume Ca in sufficient amount are very effective, especially prior to achieve maximum density of bone (around the age of 30 years). Daily drink of two glasses of milk and additional vitamin d enhance bone density in middle-aged women which previously insufficient Ca.

The ingestion of higher milk and protein results in an increase metabolism of Ca bone and the weight for approximately 16 weeks[7]. According to the *US Dietary Reference intake* (US DRIs) 2002, Ca daily needs at the age of children as much as 500-1,300 mg, while the age of 19 - 50 years to 1,000 mg, and 1,200 mg for >50 years of age. Conversely, the consumption of Ca Indonesians is currently only in the range of 254 mg per day, less than *US Dietary Reference intake* daily need of Ca per day.

Ovariectomy is an act of the dissection or technique of laparatomy for retrieval the bilateral ovary. Widely in the biomedical field, ovariectomized rats are the model of *juvenile osteopenia*[8, 9] and were able to become a model of post-menopause woman[10,11]. Arjmandi *et al*.[12] prove ovariectomy on both ovaries on experiment rat will induce osteoporosis in trabeculae jawbone because ovariectomy will stimulate the performance of osteoclast; causing the loss of bone mass in trabeculae, but does not occur at the cortical bones. Besides, the act of ovariectomy quickly inflicting menopausal symptoms without incurring a other symptom.

In ovariectomized rat, bones resorption activity was found increasing. It is in accordance with the role of estrogens against the bone. Loss of the ovary function in producing steroid sex hormones, e.g. estradiol will cause the condition of hypoestrogenis, a major lost of mass bone[13]. Hysterectomy with bilateral ovariectomy was correlated to high risk of osteoporosis[14]. Kalu *et al*.[15] and Dempster *et al*.[16] declared that ovariectomy cause the change and decrease in volume of a bone, an increasing number of osteoclast, elevated levels of enzymes and the serum of alkaline phosphatase.

Fish bone flour of *Madidihang* has high content of Ca (calcium) and P (phosphorus). Related to this, we aim to determine the dose therapy of *Madidihang* fish bone flour and $CaCO_3$ towards calcium and phosphorus content in the blood and the bone of the ovariectomy rat.

Materials and Method

Materials

The materials used in this research are the *Madidihang* fish bone flour with boiling medium of water and acetic acid, while Ca carbonate used as a comparison. The surgery of the rat used the dose of 0.3 mg (0.03 mL) mixture of Xylazine (Xylazine-20, Troy Laboratories Australia PTY Ltd) and 1.5 mg dose (0.03 mL) Ketamine (Ketamil, Troy Laboratories Australia PTY Ltd); which is administrated intraperitoneal (ip). For antibiotic administering, we use Nebacetin (Pharos, Indonesia). In vivo test used fish flour bone *Madidihang* by concentration of 0, 400, 800, and 1600 mg/g bw with the high level and solubility of Ca and P. Animals trial used 96 female wistar (*Rattus novergicus*) with age of 75 days.

Biochemistry Analysis of the Blood Serum

Prior to blood sampling, rats drugged by high doses in *anaerobic jar*. After faint, rat's blood was taken from the atrium of the heart by using 5 ml syringe. Biochemistry analysis of rat's blood serum is Ca and P content on blood by Automatic Absorbance Spectrophotometer (AAS)[17].

Histopathology of Bone Organs

In the final day treatment (31st day), all rats were dislocated on the neck. The organ of bone and skeleton were taken by necropsy after the rat dead. Further, we made flour of rat bones and analyse the Ca and P content also by AAS[17].

Results And Discussion

Ca Content in the Rat Blood

The highest value of Ca content obtained by treatment of Ca carbonate for 8.273 mg/dL and the lowest was 7.127 mg/dL of deproteinization treatment, both at the highest doses of 1600 mg/g bw/day. The second highest is on the dose of 800 mg/g bw/day with a value of 7.618 mg/dL. Statistical analysis showed the significant difference ($p<0.05$) between doses of 0, 400, 800 and 1600 mg/g bw/day (Table 1); it means that the

effect on response to treatment is observed. Similar to Firmansyah[18] that found the high doses of Ca carbonate supplements (450 mg/day) affected the process of histopathology image repairing of the femur in ovariectomized white mice. The results indicate that the treatment that was given to these rats have an effect on the content of Ca in the blood.

Table 1. The calcium levels in blood serum of rats

Dose (mg/g bw/day)	Ca blood (mg/dL)		
	Deproteinization	Non deproteinization	$CaCO_3$
0	1.740 ± 0.005^{h}	5.381 ± 0.014^{d}	2.819 ± 0.005^{g}
400	3.343 ± 0.361^{f}	4.215 ± 0.543^{e}	3.343 ± 0.361^{f}
800	5.474 ± 0.132^{d}	5.836 ± 0.465^{d}	7.618 ± 0.169^{b}
1600	7.127 ± 0.138^{c}	7.951 ± 0.097^{ab}	8.273 ± 0.032^{a}

Description: The numbers in the same row followed by different superscript letters shows significant difference ($p<0.05$).

A level of Ca blood is a primary cell's extra cation that functioned for contraction and excitation of the heart muscle and also in transmission of nerves system. A normal level of Ca in plasma is 8.5-10.4 mg/dL. Ca level of blood is 45% protein bound by plasma, especially albumin and 10% bound by anion buffer as citric and phosphate[19]. Thus, this research has not met the standards yet. We assumed it is because the experimental animals were the rat model of osteoporosis. The descent of intestinal Ca absorption and high Ca excretion through the kidney is due to the descent of 1.25 dihydroxyvitamin D_3 in the blood[20].

P Content in the Rat Blood

P is the second abundant inorganic element after Ca. P will accumulates in bone for 85%, source from milk, meat, eggs, fish, nuts and bones of fish. Although considered as essensial nutrients, excess levels of P in the body to be disserve the bone. Elevated levels of P in a serum will increase the secretion of parathyroid hormone causing the bone resorption[21,22]. Calvo[23] assess on the animals trial found that the combination of high P and low Ca decreasing the bone mass.

The highest value of P level found in the treatment of deproteinization at 1600 mg/g bw/day for 5.407 mg/dL and the lowest found in Ca carbonate treatment for 2.014 mg/dL. Whereas for doses of P: 0, 400 and 800 mg/g bw/day, the highest P content showed by the treatment of non deproteinization for 3.161 mg/dL. High P level on the blood serum of 1600 dose treatment of deproteinization shows that the higher dose treatment given the increasing value of P level. On this research, the doses of 0, 400, 800 and 1600 mg/g bw/day were significantly different ($p<0.05$) towards P content in the blood serum (Table 2).

Table 2. The phosphorus levels in the blood serum of rats

Dose (mg/g bw/day)	P blood (mg/dL)		
	Deproteinization	Non deproteinization	$CaCO_3$
0	0.727 ± 0.091^{d}	0.727 ± 0.091^{d}	0.734 ± 0.081^{d}
400	1.754 ± 1.045^{d}	0.754 ± 0.638^{d}	1.158 ± 0.139^{cd}
800	1.537 ± 0.577^{d}	3.161 ± 1.859^{bc}	2.777 ± 2.363^{d}
1600	5.407 ± 0.51^{a}	4.387 ± 0.124^{b}	2.014 ± 0.000^{cd}

Description: The numbers in the same row followed by different superscript letters shows significant difference ($p<0.05$).

Research Noor *et al.*[22] said that Ca/P ratio in the serum not always describes the same Ca/P ratio on the bone. On the osteoporosis bones which assessed by XRF, the combination of high P and Ca; as for normal bones, they found the combination of low P and Ca. On the normal bones, the process of mineralization still in the domain of regularity, thus it is strongly assumed as relation to age. When the overall mineral content increased, bone become harder and stronger, and when optimum mineralization point was exceeded then bone become weak or fragile[24, 25]. It is along with Busse *et al.*[26] who stated that the normal bone mineralization shows the homogeny pattern while in osteoporosis bone obtained sac mineralization and hypermineralization.

Ca Content in the Rat Bones

Ca content of rat bone of on three treatment and four doses of treatment, showed the highest bone Ca content in the treatment of deproteinization with 5.407 mg/g dw and the lowest in the treatment of Ca carbonate for 2.014 mg/g dw. Statistic analysis showed significant difference ($p<0.05$) between doses (Table 3).

Table 3. The calcium levels in bones of rats

Dose (mg/g bw/day)	Ca bone (mg/g dw)		
	Deproteinization	Non Deproteinization	$CaCO_3$
0	1.72 ± 0.01^{j}	1.72 ± 0.01^{j}	1.71 ± 0.05^{j}
400	3.75 ± 0.01^{g}	3.08 ± 0.01^{h}	2.82 ± 0.00^{i}
800	5.57 ± 0.02^{d}	5.12 ± 0.01^{e}	4.08 ± 0.00^{f}
1600	8.29 ± 0.02^{a}	7.52 ± 0.04^{b}	7.04 ± 0.03^{c}

Description: The numbers in the same row followed by different superscript letters shows significant difference ($p<0.05$).

Individual bone mineral density increased in post-menopause, thus need high doses of Ca[27]. The atoms of bone mineral will form the pattern of geometric as matrix which determine the image of bone microstructures. According to science material approach, the strength of bone structure is not only achieved by high Ca mineral, but also other minerals which are arranged in the geometric pattern of bone[22].

According to O'Loughlin and Morris [28], there is a link between the retention of Ca with the accumulation of minerals in bones, while according to Wood [29] retention of Ca reflects the balance between the processes of formation and bone resorption during bone remodelling process. Higher retention of Ca shows the higher formation of bone compared to bone resorption, and vice versa. The image of physical and metaphysical histopathology on the distal femur of normal rat show physical osteogenic zone, while spicules of trabeculae in the metaphysic were normally shaped, and the bone marrow cavity is dominated by hematopoietic tissue.

P Content in the Rat Bones

In the dose of 1600 mg/g bw/day, the highest P content obtained from deproteinization treatment for 2.224 mg/g dw and the lowest P content is from Ca carbonate treatment for 1.475 mg/g dw. Whereas at doses of 800 mg/g bw/day, the highest P content assessed in the non deproteinization treatment for 1.370 mg/g dw and the lowest in $CaCO_3$ treatment for 1.111 mg/g dw (Table 4). For bones, P diet can be detrimental. The harm caused by P ability to trigger a temporary decline of Ca serum, increase the parathyroid hormone, and continuous increasing parathyroid hormone will enhance the bone resorption [21]. The result suggests that the treatment with four therapeutic doses of *Madidihang* bones given to ovariectomized rat model were significantly affect the P content in the bone of rat.

Table 4. The phosphorus levels in bone of rats

Dose (mg/g bw/day)	P bone (mg/g dw)		
	Deproteinization	Non deproteinization	$CaCO_3$
0	0.727 ± 0.091^{d}	0.727 ± 0.091^{d}	0.734 ± 0.081^{d}
400	0.709 ± 0.071^{f} 1.323	0.471 ± 0.004^{d}	1.368 ± 0.044^{d}
800	$\pm 0.025^{d}$	1.370 ± 0.045^{d}	1.111 ± 0.020^{e}
1600	2.224 ± 0.103^{a}	2.010 ± 0.12^{b}	1.475 ± 0.009^{c}

Description: The numbers in the same row followed by different superscript letters shows significant difference ($p<0.05$).

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