

Mangosteen Extract Inhibits LPS-Induced Bone Resorption by Controlling Osteoclast

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Abstract

This study examined the efficacy of fruit hull extracts of *Garcinia mangostana* L., in conserving bone structure against LPS-induced bone destruction.

Bone destruction was established by injecting mice calvarial bone with LPS. We then treated the LPS-infected calvarial bone with *Garcinia mangostana* L. extract and alpha-mangostin, respectively, and examined the bone destruction level and osteoclast number after 5 days.

The results showed the significant reduction of bone destruction in *Garcinia mangostana* L. extract-treated group and in alpha-mangostin treated group. Furthermore, the osteoclast surface area in all treated groups were also significantly reduced. The group treated with *Garcinia mangostana* L. extract showed the best result in preventing bone destruction compared to the alpha mangostin treated group.

Our results demonstrated the therapeutic effect of *Garcinia mangostana* L. extract as the traditional medicaments against the exuberant osteoclast resorbing activity that hence has the potential to preserved bone quality. Our results demonstrated the therapeutic effect of *Garcinia mangostana* L. extract as the traditional medicaments against the exuberant osteoclast resorbing activity that hence has the potential to preserved bone quality.

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Introduction

Bone is a hard tissue that undergoes continuous dynamic remodeling process in the form of resorption by osteoclasts and formation by osteoblasts. The process of bone remodeling depends on the activity of osteoclast to resorb bone matrix, and osteoblasts, which serves to synthesize new bone matrix. In normal conditions, bone resorption and synthesis is in a balanced state, so that the amount of bone resorbed is always equal to the amount of newly formed bone.¹ This balance is achieved and regulated by the activity of various systemic hormones (eg. parathyroid hormone, vitamin D and other steroid hormones) and local mediators (eg. cytokines,

growth factors). In contrast, aging, metabolic bone disease, a condition with increased or decreased mobility, therapeutic intervention and other conditions, are resulting in an imbalance of bone remodeling.² Imbalance in bone remodeling will affect the bone mass, which in turn trigger abnormal pathological conditions.³

Osteoclast is a multinucleated cell, which is able to degrade organic and inorganic bone matrix. A wide range of pathological condition accompanied by bone loss, such as that found in patients with osteoporosis, is known to have closely association with excessive activity of osteoclasts. Therefore, knowledge related to mechanisms of differentiation and activity of osteoclasts in bone resorption is very important.^{4,5}

Osteoclast differentiation is supported through contact between cells by mesenchymal cells (bone marrow stromal cells or osteoblasts). Previous study revealed that osteoclastogenesis requires contact with osteoblast precursor molecules that induce osteoclast differentiation.⁶

Osteoclast differentiation molecule that

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was originally called osteoclast differentiation factor (ODF), is in the present known as RANKL. In addition, M-CSF, which is secreted by osteoblasts, induces signals that play a role in the survival of osteoclasts.^{7,8}

Understanding the central mechanisms underlying the formation and function of osteoclasts has greatly contributed to the development of therapies aimed to preserve bone mass. First line treatment for diseases-involving excessive bone loss, such as osteoporosis or cancer-induced bone disease, is antiresorptive therapy, usually with the use of bisphosphonates. The use of bisphosphonate drugs was proven to work effectively inhibits the activity of osteoclasts but induces osteonecrosis of the jaw and bone loss.⁹ Thus, a new material that is able to reduce the resorptive activity of osteoclasts is necessary in the treatment of bone disorders which targeting osteoclast differentiation and function.

One potential candidate to control excessive activity of osteoclasts is mangosteen (*Garcinia mangostana* L.). Mangosteen has been extensively used as a traditional medicine for centuries specifically in Southeast Asia (Indonesia, Thailand, Malaysia) and has a long history of use in traditional medicine as well as in modern medicine.¹⁰ Extensive studies related to the effect of the bioactive components of this fruit had been performed long time ago by various groups of researchers.^{11,12} This ethnomedicinal fruit has been well documented for the treatment of diarrhoea, dysentery, inflammation, and many others.^{13,14}

In the recent years, studies about mangosteen have revealed the potency of mangosteen in a wide range of medicinal properties. The fruit has benefits as an anti-inflammatory, antioxidant, antibacterial, antifungal and possesses cytotoxic activity.¹⁵⁻¹⁹

The bioactive substances contained in mangosteen have the potential to inhibit proliferation of some cells and induce apoptosis in tumor cells, especially bone tumors.¹⁹

Moreover these substances potentially inhibit several anti-inflammatory mediators, such as interleukins, which have previously been known to enhance the differentiation and activity of osteoclasts.^{4,20} Thus, it is expected that *Garcinia mangostana* L. has the similar effect in osteoclasts to inhibit excessive bone resorption.

In this study, we investigated the effect of

the administration of *Garcinia mangostana* L. extract and alpha-mangostin respectively to the destructive bone induced by the application of lipopolysaccharide (LPS) and evaluate whether *Garcinia mangostana* L. and its compounds are potential to be an alternative therapy as the traditional medicine for bone loss.

Materials and methods

This study is an experimental *in vivo* study using mice calvarial bone histological analysis to determine the effect of *Garcinia mangostana* L. against *in vivo* bone destruction. All of the animal experiments were performed with the approval of the Ethical Committee of Faculty of Dentistry, Universitas Indonesia and conformed to relevant guidelines and laws in accordance with the US guidelines (NIH publication #85-23, revised in 1985).

Plant Materials

Garcinia mangostana L. was obtained through production center in Leuwiliang-Bogor regency (06° 60' S, 106° 60'E), West Java, Indonesia. The specimen was authenticated and processed by Prof. Sobir at the Center for Tropical Horticulture Studies, Bogor Agriculture University, Indonesia (voucher specimen number, 2046/Kpts/SR.120/5/2010).

Sample preparation

Inner *Garcinia mangostana* L. hull was dried, cut into small pieces (0.5kg) and extracted using ethyl acetate (EtOAc) at a temperature of 50°C. Dilution of alpha-mangostin and *Garcinia mangostana* L. whole extract (WE) were using 0.9% NaCl, divided into four treated groups, 250 and 500µg/ml WE, 125 and 250µg/ml alpha-mangostin.

LPS-induced bone destruction

LPS-induced bone destruction was performed as previously described.²¹ Briefly, local injection of lipopolysaccharide (LPS; Sigma-Aldrich) was performed on mice calvarial bone with a dose of 25mg/kg body weight. After 24 hours, the *Garcinia mangostana* L. whole extract, alpha-mangostin or saline water-only, was injected in the same area. Calvarial bone were then analyzed by histological examination.

Samples

The subjects of the experiments were thirty male mice (*Mus musculus*) strain Swiss Webster aged 6-8weeks. The sample size was calculated using the Federer formula: $(n-1)(t-$

1)>15. For the group number by 5, the optimal amount of sample is 6 mice per group.

Analysis of bone damage

Bone damage was analyzed by calculating the area of bone erosion in histological preparations using the ImageJ software. Results between groups were analyzed statistically to see the significance of differences between groups.

Osteoclasts analysis

Number of osteoclasts were counted on the histological preparations stained with TRAP, a specific staining to identify osteoclasts. Osteoclast cell surface area was then measured by ImageJ software. Results between groups were analyzed statistically to see the significance differences between groups.

Statistical analysis

Statistical analysis was performed using the Mann-Whitney Test. (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$, n.s., not significant, throughout the paper).

Results



Figure 1. Calvarial bone of mice after injection of (A) saline water and (B) lipopolysaccharide (LPS). Red circle indicates the inflamed area after LPS injection.

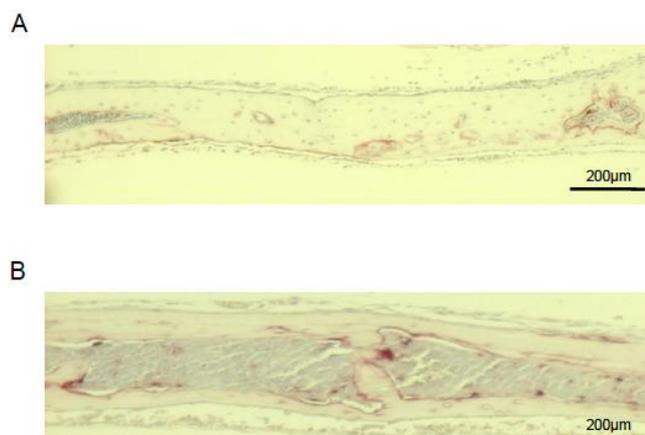


Figure 2. Histological preparation of calvarial bone of mice after injection of (A) saline water and (B) lipopolysaccharide (LPS).

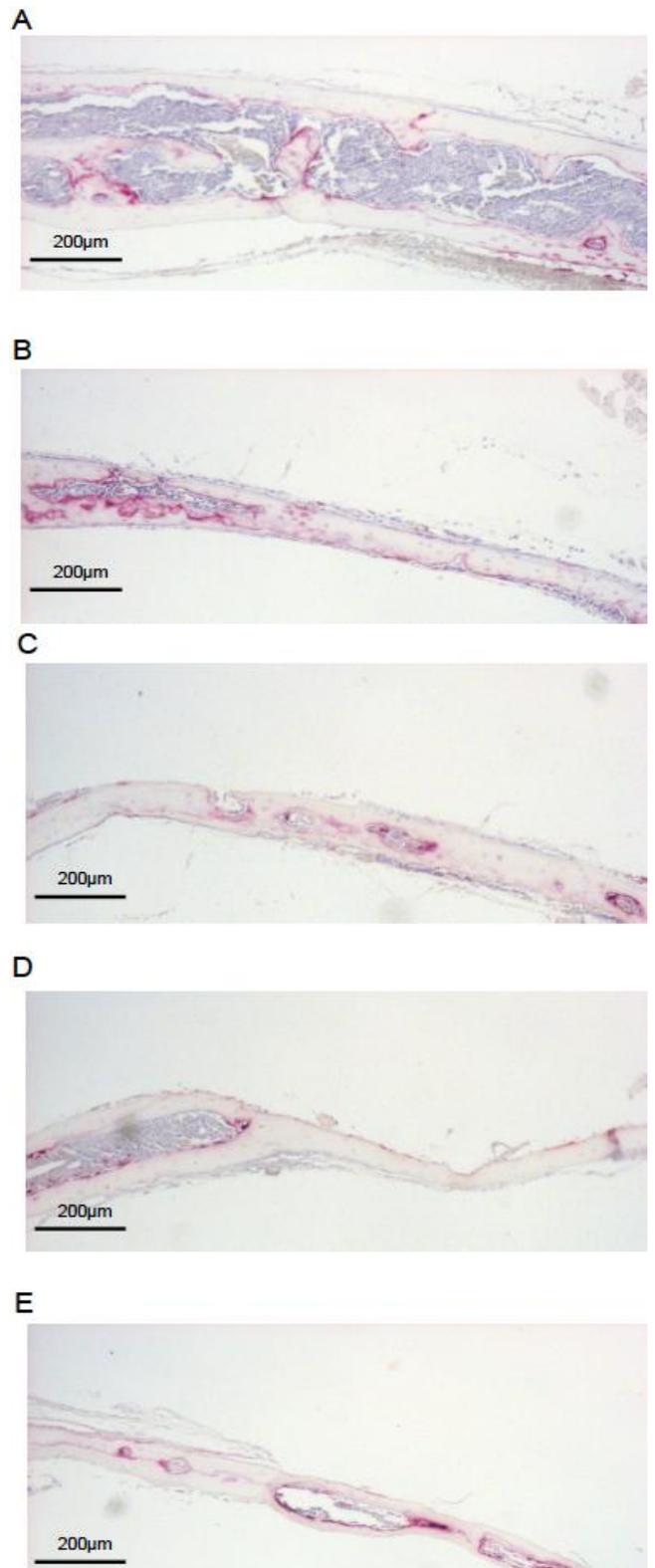


Figure 3. Histological preparation of calvarial bone of mice after injection LPS and followed by the treatment of (A) saline water, (B) whole mangosteen extract 250µg, (C) whole mangosteen extract 500µg, (D) alpha mangostin 250µg and (E) alpha mangostin 125µg.

LPS application performed on calvarial bone showed redness inflammation sign, at the application area (Figure 1B) compared to the control one (Figure 1A). Histological analysis also confirmed the bone destruction triggered by the LPS application on the calvarial bone (Figure 2B), while the control bone remained intact (Figure 2A).

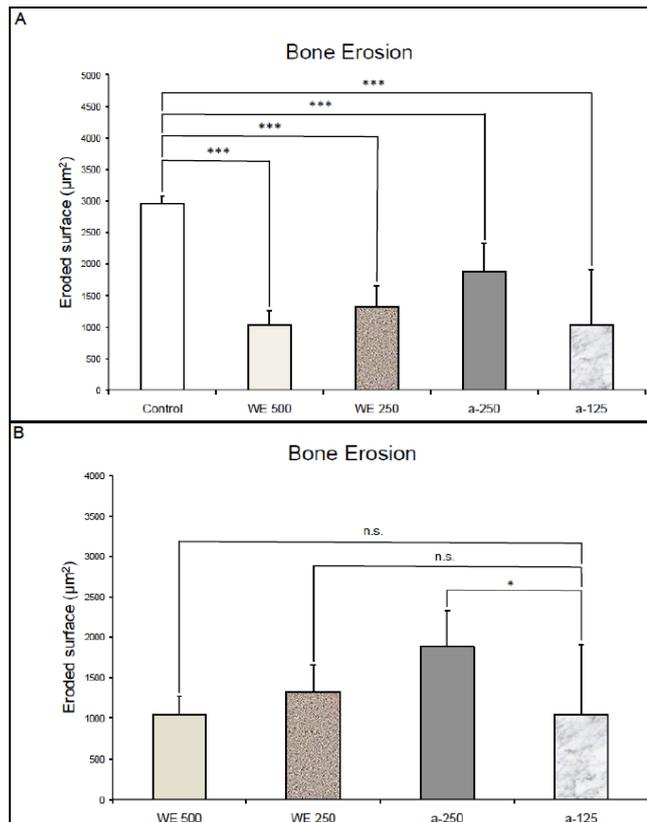


Figure 4. Statistical comparison of bone erosion of control, whole mangosteen extract 500 and 250µg (WE 500/250), and alpha mangostin 250 and 125µg (a-250/125). (*p<0.05, **p<0.01, ***p<0.005, ns, not significant)

Five days after LPS and medicaments application, mice were sacrificed and calvarial bone were isolated. Histological preparations were made, followed by TRAP staining to identify osteoclasts. Analysis of the results showed bone destruction decreased in all treatment groups compared to controls (p<0.05) (Figure 3A-E) (Figure 4A).

Group treated with 500µg *Garcinia mangostana* L. whole extract (WE 500) showed the highest decreased bone damage compared to the other treatment groups (Figure 4B). In addition, osteoclasts surface area of all treated groups declined significantly compared to

controls (p<0.05) (Figure 5A). In comparison to control and the other treatment groups, the group treated with WE 500 showed the greatest reduction of osteoclast surface area (p<0.05) (Figure 5B).

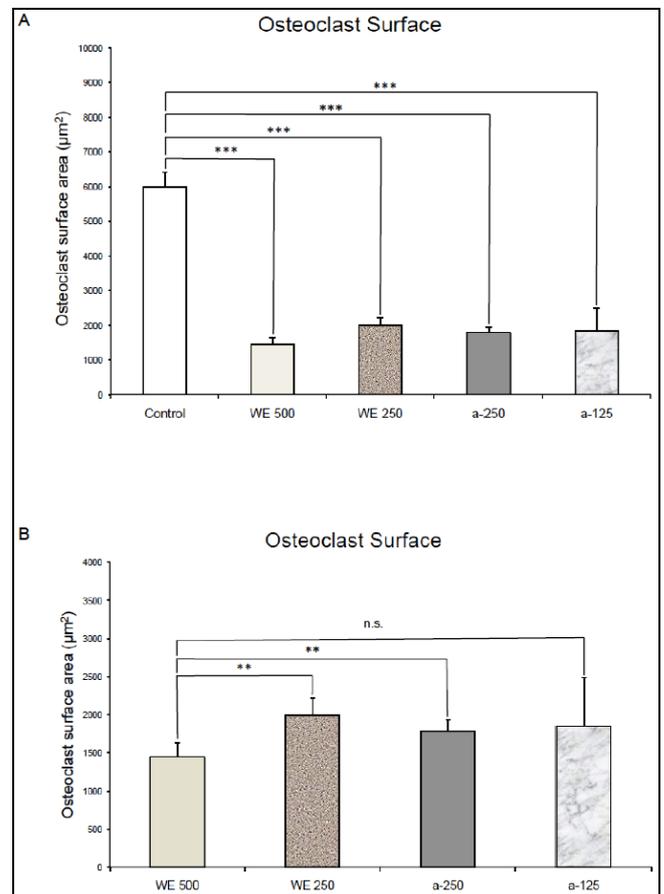


Figure 5. Statistical comparison of osteoclast surface area of control, whole mangosteen extract 500 and 250µg (WE 500/250), and alpha mangostin 250 and 125µg (a-250/125). (*p<0.05, **p<0.01, ***p<0.005, ns, not significant)

Discussion

Bone resorption is tightly regulated by osteoclast, which is controlled by a wide range of cytokines involved in its differentiation, activity and survival. Hence, the extent of bone destruction inevitably depends on the quantity of osteoclast, the potent ability of the individual osteoclast to release resorbing agent and survival rate of osteoclast against apoptosis.^{4,6}

The mechanism of the bone defect inhibition by *Garcinia mangostana* L. extract in this study is tightly regulated by the suppression of osteoclast number, as shown by the decreased number of osteoclast in ameliorated

bone destruction. The present study showed the treatment with the *Garcinia mangostana* L. extract resulted in significant reduction of bone erosion and osteoclast surface area. In addition, the LPS-infected calvarial bone administered with alpha-mangostin in two concentrations (250 and 125µg/ml) also showed significant reduction of bone destruction compared to control. Furthermore, the possible mechanism of osteoclast regulation by *Garcinia mangostana* L. extract and alpha-mangostin may act through several pathways.

Osteoclast bone resorption is proven to be activated by inflammatory stimuli, shown in several inflammatory diseases accompanied by increased osteoclast number and activity. In the previous study, alpha-mangostin compounds demonstrated effectiveness as medicament of inflammatory diseases by inhibiting the production of nitric oxide, TNF-α and IL-8 secretion in various cell line.^{22,23} All of these inflammatory mediators have also been known to enhance the differentiation and activity of osteoclasts.^{4,20} For example, nitric oxide production has been reported to be essential in osteoclast survival. Addition of carboxy-PTIO, a nitric oxide scavenger, showed inhibition of bone resorption and activation of caspase 3 to induce osteoclast apoptosis.²⁴

Alpha-mangostin was found to be the major xanthone found in *Garcinia mangostana* L. among other type of xanthenes. Extensive investigation into the role of alpha-mangostin in cell metabolism revealed the important role of this xanthone as anti-inflammatory, antitumor, inducer of apoptosis and anti-proliferative on cancer cells.²⁵⁻²⁸ A study in macrophage cells proved that alpha-mangostin reduced nitric oxide production and potentially inhibited inflammation.²³

Although both groups (administered with *Garcinia mangostana* L. extract and alpha-mangostin, respectively) exhibited significant inhibition in bone destruction, the groups treated with whole extract of *Garcinia mangostana* L. showed superior inhibition against bone destruction as compared with the other groups. These findings suggested that *Garcinia mangostana* L. compound(s), other than alpha-mangostin were indispensable in preserving bone microstructure, but the precise mechanism underlying osteoclasts regulation by *Garcinia mangostana* L. extract remains to be elucidated.

Garcinia mangostana L. comprises various compounds found in its fruit hull that involves in body metabolism, including xanthenes, vitamins and other bioactive compounds.^{10,20} In a study by Fu et al, it was revealed that isogarcinol, a compound extracted from *Garcinia mangostana* L., attenuated nitric oxide production and inhibited NF-κB expression, an important transcription factor in osteoclast differentiation.²⁹

Isogarcinol was also found to be a strong inhibitory molecule of calcineurin.³⁰ Calcineurin is a molecule tightly involved in osteoclast differentiation signaling pathways of which the disruption of this molecules will result in inhibition of osteoclast differentiation.^{31,32}

Through this study, the authors suggest the use of a 125µg/ml dose whole extract *Garcinia mangostana* L. as the optimum dose for local application, so as to avoid unwanted effects from the use of high doses of *Garcinia mangostana* L. extract that may occur directly or indirectly.³³ Further studies regarding the most appropriate dose of *Garcinia mangostana* L., and research on the side effects of short-term and long-term application of alpha-mangostin and *Garcinia mangostana* L. extract must be performed in order to find the appropriate dosage in treating bone diseases.

In summary, these findings demonstrate a role of *Garcinia mangostana* L. in controlling osteoclast bone-resorbing activity that leads to the protection of bone microstructure. The extensive study of *Garcinia mangostana* L. inhibition of bone destruction is urgently needed to reveal the exact mechanism of how *Garcinia mangostana* L. protects bone loss. Furthermore, each major bioactive compounds of *Garcinia mangostana* L. needs to be investigated meticulously to provide information as the basis to establish new therapeutic agent against bone loss.

Conclusions

Decreased in the number of osteoclasts and ameliorated bone destruction observed in LPS-infected calvarial bone after the application of *Garcinia mangostana* L. extracts, demonstrates the potential role of *Garcinia mangostana* L. extract in protecting bone damage caused by the increased number of osteoclasts. Further investigation is needed to comprehend the mechanisms of osteoclast

suppression by *Garcinia mangostana* L., which in turn is expected to be an alternative traditional medicament in preventing bone deterioration.

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Declaration of Interest

The authors report no conflict of interest.

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