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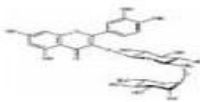


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Bacteriological Quality of Water Supplies Utilized in Abattoirs Located in Benin City, Ekpoma and Auchi



Viability of α -mangostin and Lipopolysaccharide (LPS) in 7F2 Cells

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ABSTRACT

Based on data from RISKESDAS in 2018, the incidence of tooth extraction in Indonesia is 19%. In post-extraction of teeth, the gingival tissue around the extraction area is inflamed as well as a decrease in the dimensions of the alveolar ridge. Socket preservation is performed to reduce the possibility of bone resorption and bone formation. Alpha(α)-mangostin from mangosteen peel extract (*Garcinia mangostana* Linn.) is expected to accelerate post-extraction healing so that implant placement can be more adequate. The study analyzed the viability of α -mangostin and lipopolysaccharide on 7F2 cells lines. Microtetrazolium (MTT) assay against 7F2 cell culture was used. The correlation between concentration and viability of Lipopolysaccharide (LPS) and α -mangostin was interpreted by statistical analysis. The viability of the 7F2 cell culture increased in line with the increase in the concentration of α -mangostin and the optimal concentration of 8.25 g/mL with the highest percentage of living cells at 132.120%. α -mangostin can increase the viability of 7F2 cell culture and it has the potential to be used as an alternative material to reduce inflammation after tooth extraction.

Keywords: α -mangostin, Lipopolysaccharide, MTT assay, socket preservation, medicine.

Introduction

Dental and oral health problems that are often encountered are tooth loss due to caries and periodontal disease. Based on Riset Kesehatan Dasar Nasional (RISKESDAS) in 2018,¹ the incidence of tooth loss in Indonesia is 19%. Tooth loss can affect aesthetic appearance, interfere with the masticatory system, and affect the comfort of speech.² According to the 2010 RISKESDAS results, the incidence of tooth extraction in Indonesia was 79.2%. The healing process of the alveolar ridge after tooth extraction goes through a long process and it can cause a decrease in the dimensions of the alveolar ridge by almost 50% of its original width.³ According to radiographic studies, the alveolar ridge generally disappears in the first 90 days following the extraction.⁴ Socket preservation, a treatment that can assist make up for the degradation of the alveolar bone, is required to get around this and maintain bone volume after tooth extraction. However, socket preservation procedures are still needed to reduce the possibility of bone resorption and accelerate bone formation to increase the success of dental implants.⁵

Garcinia mangostana is a tree native to Southeast Asia whose fruit is known as mangosteen. Extracts of mangosteen fruit have been used in traditional medicine. The health-promoting properties of mangosteen peels are related to a family of compounds called xanthenes. These hydrophobic compounds have tricyclic aromatic ring systems with various mixtures of isoprenyl, hydroxyl, and methoxy substitutions. Alpha (α)-mangostin is the most abundant xanthone in mangosteen peel.

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In vitro studies have consistently demonstrated that xanthenes have antioxidant, antiproliferative, proapoptotic, antibacterial, anti-inflammatory, and anticancer activities. Anti-inflammatory and anticancer activity has also been demonstrated in rodents.⁶ In this study, we used 7F2 cell culture which is a precursor cell of osteoblasts derived from the bone marrow of *Mus musculus* as a medium for testing the viability of α -mangostin derived from Mangosteen (*Garcinia mangostana*) and lipopolysaccharide (LPS). In overcoming inflammation after implant placement, α -mangostin was chosen because it has antibacterial properties with a fast killing ability and broad clinical aspects.^{7,8} The MTT assay was used to determine the viability of these cells.

Material and Method

This study has been approved by the ethical health committee of the Faculty of Dental Medicine, Universitas Airlangga for animal laboratories with number of certificate 786/HRECC.FODM/XII/2019. This study design uses the laboratory experiment with a post-test-only control group design.

Samples and cell culture

The α -mangostin was obtained from Sigma Aldrich, Inc., St. Louis, Missouri (M3824). The component used in this study was 7F2 cell culture which is a precursor cell of osteoblasts derived from the bone marrow of *Mus musculus* (Mouse C57BL/6) the number ATCC CRL-12557 was purchased at the American Type Culture Collection (Manassas, America).—The lipopolysaccharide (LPS) used for the experiment is from Sigma Aldrich, Inc., St. Louis, Missouri (L2630). LPS comes from *Escherichia coli* O111:B4. LPS extract was dissolved in sterile water then lyophilized and weighed.

MTT assay

Examination of the viability of the cells was determined using the MTT assay method. Spectrophotometry which if the measurements are correct, the absorbance value will be directly proportional to the number of living cells used. The MTT reagent, Invitrogen, Massachusetts, USA (M6494) was used. Samples of LPS were divided into six groups namely, Group 1. 10 g/mL, Group 2. 20 g/mL, Group 3. 30 g/mL, Group

4. 40 g/mL, Group 5. 50 g/mL, Group 6. 60 g/mL. Each well was administered DMEM (11885084, Sigma–Aldrich, Inc., St. Louis) + 10% FBS (10270106, Life Technologies Limited, Paisley, UK) + 1% Penicillin-Streptomycin (15140148, Sigma–Aldrich, Inc., St. Louis, Missouri) and incubated in an incubator at 37°C for 24 hours. After incubation, the reagent was added to each well and then again incubated for 2 hours. The absorbance value was measured at a wavelength of 450 nm using an epoch microplate reader (Biotek, Santa Clara, USA). The α -mangostin samples were divided into six groups, namely, Group 1. 2.5 g/mL, Group 2. 3.75 g/mL, Group 3. 5 g/mL, Group 4. 6.25 g/mL, **Group 5.** 7.5 g/mL, and **Group 6.** 8.25 g/mL. Each well was given the same treatment as the lipopolysaccharide sample and then the absorbance value was measured using an epoch microplate reader 540nm.

Results and Discussion

The results of the absorbance measurement of the 7F2 cell culture test using the MTT method using 3 replicates from various concentrations of α -mangostin and LPS are presented in Tables 1. and 2.

LPS had the lowest average absorbance value against 7F2 cell culture at a concentration of 60 g/mL with concentrations lower than 60 g/mL an increase in absorbance was seen. This suggests that the viability of the 7F2 cell culture will decrease with increasing LPS concentration.

Alpha-mangostin which is derived from the pericarp of *Garcinia mangostana* L. has the highest average absorbance value against 7F2 cell culture at a concentration of 8.25 g/mL with a concentration lower than 8.25 g/mL seen a decrease in absorbance. This means that the viability of the 7F2 cell culture will increase in line with the increase in

the concentration of α -mangostin and the optimal concentration of 8.25 g/mL with the highest number of living cells of 132.120%.

Alpha-mangostin has received great attention because of its health-promoting benefits which include antibacterial and anti-inflammatory properties.⁹ The anti-inflammatory properties of α -mangostin have been demonstrated in in vitro cellular models (such as mouse or human macrophages, adipocytes, and human adipocytes exposed to macrophage-conditioned medium (CM)) and in in vivo animal models (such as carrageenan-induced paw edema in ICR mice and collagen-induced arthritis in mice. Alpha-mangostin was reported to improve glucose uptake and inhibit adipocyte differentiation.¹⁰

Alpha-mangostin has been reported to have inhibitory activity against many microorganisms, including *P. acnes*, *S. epidermidis*, and *S. aureus*, and also has anti-inflammatory activities.¹¹ In addition, α -mangostin compounds have anti-inflammatory activity by inhibiting the production of nitric oxide, TNF- α , and IL-8 secretion known to cause bone resorption and inhibit bone formation.¹² The activity of osteoclasts in bone resorption and the activity of osteoblasts in the matrix of synthesizing new bone are things that affect the remodeling process of bone. Under normal conditions, bone resorption and synthesis occur in balance.¹³ Previous studies have shown that α -mangostin can inhibit osteoclastogenesis, the process is carried out by inhibiting COX-2 so that pro-inflammatory cytokines (IL-1, TNF- α) and PGE-2 are decreased. In addition, α -mangostin can inhibit bone resorption by interfering with the binding process of RANKL to RANK on the surface of preosteoblasts so that the NF- κ B signaling pathway will be disrupted whereas NF- κ B is useful for osteoclast development.^{14,15} Alpha-mangostin also showed a protective effect on Lipopolysaccharides (LPS)-induced inflammatory bone loss by inhibiting the secretion of IL-1 β , IL-6, NO, and COX-2.¹⁴

Table 1: Concentration and Absorbance in Viability Test of α -mangostin (μ g/mL)

Replication	Medium Only	Sel Only	2.5	3.75	5	6.25	7.5	8.25
1	0.074	0.401	0.341	0.365	0.373	0.404	0.414	0.409
2	0.086	0.403	0.386	0.423	0.432	0.464	0.425	0.487
3	0.076	0.369	0.440	0.422	0.416	0.376	0.490	0.578
Total	0.236	1.173	1.167	1.210	1.221	1.244	1.329	1.474
Mean	0.079	0.391	0.389	0.403	0.407	0.415	0.443	0.491
Total % of Living Cells			99.360	103.948	105.122	107.577	116.647	132.120

Table 2: Concentration and Absorbance in LPS Viability Test (η g/mL)

Replication	Medium Only	Sel Only	10	20	30	40	50	60
1	0.074	0.401	0.211	0.189	0.208	0.184	0.181	0.207
2	0.086	0.403	0.271	0.262	0.235	0.219	0.222	0.202
3	0.076	0.369	0.230	0.235	0.229	0.247	0.239	0.226
Total	0.236	1.173	0.712	0.686	0.672	0.650	0.642	0.635
Mean	0.079	0.391	0.237	0.229	0.224	0.217	0.214	0.212
Total % of Living Cells			50.806	48.031	46.537	44.190	43.336	42.589

Alpha-mangostin can also increase bone production by inducing the production of TGF- β 1. TGF- β 1 is an inflammatory cytokine that plays a role in the process of bone remodeling and resorption by stimulating protein matrix synthesis. In addition, TGF- β 1 is well known for its function in bone formation/osteogenesis and exhibits versatile regulatory functions in the body. During the bone resorption process carried out by osteoclasts, TGF- β 1 is released from the bone matrix. Activated TGF- β 1 will carry mesenchymal precursor cells to bone resorption via the SMAD signaling pathway. After TGF- β 1 is induced, the SMAD and p38 MAPK pathways converge in the Runx2 gene to control the differentiation of mesenchymal precursor cells. The relationship between Runx2 and TGF- β 1 activated SMAD plays an important role in osteogenesis.^{16,17}

LPS are characterized by molecular sequences that are responsive to the innate immune system. Initiate a strong immune response, signaling a premonitory bacterial infection to the body.^{18,19} LPS molecules are thermostable and generate powerful pro-inflammatory stimuli for the mammalian immune system. Furthermore, LPS is also a well-recognized biomarker because it plays a central role in host-pathogen interactions that facilitate the infection process.²⁰ In this research, LPS is used as an agent to induce inflammation in the process of osteogenesis.

A viability test was conducted to determine the potential of α -mangostin in overcoming the inflammatory reaction of the 7F2 cell culture that had been given LPS. LPS can activate the innate immune system, such as macrophages, neutrophils, and pro-inflammatory factors (IL-1 β , TNF, MMPs, and free radicals) so that they can trigger inflammatory reactions in tissues.^{21,22} The results obtained show that the viability of the 7F2 cell culture will increase in line with the increase in the concentration of α -mangostin and the optimal concentration of 8.25 g/mL with the highest number of living cells of 132.120%. However, replication 1 with a concentration of 8.25 had a lower absorbance value than a concentration of 7.5, replication 2 with a concentration of 7.5 had a lower absorbance value than a concentration of 6.25, and replication 3 with a concentration of 3.75-6.25 has a lower absorbance value than the concentration of 2.5.

Conclusion

The addition of α -mangostin from mangosteen peel extract (*Garcinia Mangostana*) and LPS to 7F2 cell culture which is a precursor cell of osteoblasts derived from the bone marrow of *Mus musculus* can increase cell viability.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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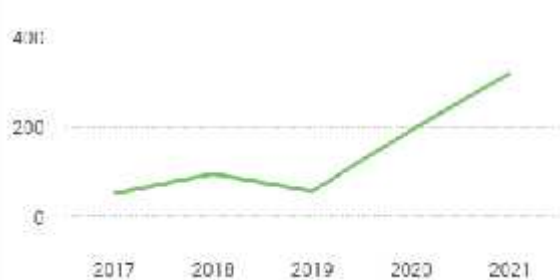


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Category	Rank	Percentile
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