Expression of Stromal-Derived Factor-1 (Sdf-1), Cxcr4 and Vascular Endothelial Growth Factor (Vegf) after Induction of Mangosteen Peel Extract (Garcinia Mangostana. L) in Mesenchymal Stem Cells Culture

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Abstract

Stem cell therapy is a promising therapy for regeneration. However, weak engraftment and lack of survival was unavoidable when stem cell being transplanted. *Garcinia mangostana. L* Peel may possess beneficial active compound to enhance homing factor of stem cells. C-X-C Motif Receptor-4 (CXCR4), Stromal Derived Factor-1 (SDF-1) and Vascular Endothelial Growth Factor (VEGF) play an important role in stimulating endogenous Bone Marrow Mesenchymal Stem Cells (BMMSCs) mobilization.

The aim of this research to find out whether mangosteen (*G. mangostana. L*) peel extract can induce the expression of a CXCR4, SDF-1 and VEGF in BMMSCs culture.

BMMSCs were taken and isolated from femur of male Wistar rats (*Rattus novergicus*). BMMSCs were cultured then added with mangosteen (*Garcinia mangostana. L)* peel extract. In this study, the culture of MSCs divided into 2 groups, group 1: added *G. mangostana. L* peel extract and group 2: without *G. mangostana. L* peel extract. The examination of SDF-1and CXCR4 expression by using enzyme linked immunosorbent assay (ELISA), meanwhile the VEGF expression by using Immunocytochemistry. Statistical analysis was test using Levene test for variance homogeneity and normality test with the Saphiro-Wilk test. If the data is normally distributed then a different test is performed using the t-test ($p<0.05$).

There was a significant difference in the expression of SDF1-CXCR4, and VEGF ($p<0.05$) in BMMSCs cells culture between two groups ($p<0.05$). Conclusion: *G. mangostana* peel extract is able to induce the expression of a number of proteins, growth factors and chemokines, such as VEGF, CXCR4, and SDF-1.

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Introduction

Stem cell therapy is an alternative approach in the treatment of various diseases including xerostomia caused by damage to the salivary glands as a side effect of radiation therapy.[1,2] At present the main focus of stem cell therapy is stem cell transplantation. Weak engraftment and lack of survival when stem cells are transplanted are one of the main weaknesses of this therapy.[2] It is important to optimize the potential of endogenous stem cells in the process of cell regeneration, but cell-based therapies also necessitate time- and money-consuming *in vitro* cell multiplication techniques.[3] previous study have shown the therapeutic potential of endogenous stem cells by promoting stem cell genesis and inducing endogenous stem cells to move to the sites of injury.[4] The endogenous stem cells in the body is to play an important role in repairing tissue that is injured or degenerative. Several studies suggest that the human body has a mechanism to mobilize bone marrow mesenchymal stem cells (BMMSCs) naturally to various areas of the body and contribute to tissue regeneration and repair.[5] Several chemokines

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and growth factors known to play an important role in stimulating endogenous BMSC mobilization are C-X-C Motif Receptor-4 (CXCR4), Stromal-Derived Factor-1 (SDF-1), and Vascular Endothelial Growth Factor (VEGF).[6] One of the most effective stimulators of angiogenesis is vascular endothelial growth factor (VEGF). When VEGF binds to VEGF receptor 1 (VEGFR1), the receptor's tyrosine kinase domain causes angiogenesis (TK).[7] It has been established that stromal-derived factor-1 (SDF-1) is an essential component in tissue regeneration. SDF-1 is primarily produced by MSCs and injured tissues in the regeneration microenvironment. SDF-1 not only functions as a critical paracrine factor derived from MSCs that promotes wound healing but also increases the effectiveness of MSC paracrine in MSC paracrine-mediated tissue repair.[8]

Some of the herbal ingredients are known to have enormous potential and pharmacological activities, one of which is the mangosteen (*Garcinia Mangostana L*).[9] Xanthones as the main compound in mangosteen have been reported to have very high anti-inflammatory and antioxidant effects that can stimulate some growth factor.[1,10] Flavonoid chemicals are also present in mangosteen (*G. Mangostana L)* peel. Flavonoids contribute to the release of tumor growth factor-β (TGF-β), which encourages the migration and proliferation of fibroblasts to the wound as well as the production of an extracellular matrix.[10] AKT, protein kinase C, and mitogen-activated protein kinase may all be triggered by the flavonoid compound xanthone, which significantly boosts the proliferation of BMSCs.[9] This research aim to investigate an in-vitro test of mangosteen (*G. Mangostana L)* peel extract in stem cell culture to find out whether mangosteen peel extract can induce the expression of some chemokines and growth factors including CXCR4, SDF-1, and VEGF which have important role in mobilizing endogenous stem cells to the injury area.[11]

Materials and methods

Preparation of mangosteen peel extract

Mangosteen *(G. mangostana L.*) peel, was bought from the Blitar Botanical Garden. The peel is then washed of dirt and other plants before being dried outside away from direct sunshine. A grinding device is used to smooth out the dried material, and a powder sieve is used to sift it. A sealed container is used to keep the resulting powder. The powder is then given a solvent to facilitate contact with the plant's active components and improve the extraction.[2,10]

The powdered mangosteen (*G. Mangostana L)* peel was then macerated in a methanol solvent for 24 hours in a closed container left at room temperature while stirring frequently to get the liquid extract. After passing it through a Buchner filter, the liquid extract is then accommodated. A fresh solvent is used to macerate the resulting particle. To acquire the mass of viscous extract, the maceration's output is collected and rotated evaporated at low pressure until it can no longer evaporate. Dry methanol extract is the remaining solvent in the viscous extract that evaporated in the resulting acid cabinet.[2,8]

Mesenchymal Stem Cells Culture

The bone marrow samples were dissolved in three equal portions of MSC growth media and then uniformly distributed across three 10 cm culture dishes. Each dish contained 10 ml of diluted aspirate, which was kept for 4-5 days in an incubator (made by ThermoScientific Heraeus, USA) with 5% CO2 at a constant temperature of 37°C. The media was changed every three to four days, and ultimately the red cell and detached, unidentified cell contamination was diluted and rinsed out. Within 5-7 days, little MSC colonies of fibroblast cells were connected and visible. Small colonies might be seen after 12–14 days. In this situation, cells were rinsed with α-MEM devoid of serum before being sub-cultured. After the addition of 5 ml of 0.05% trypsin, cells started to be removed from the substrate after a short period. Examine the findings with a microscope (JEOL, JSMT1000, Scanning Microscope, Japan). As long as the entire cells had not been given, the trypsin/ethylenediaminetetraacetic acid solution (Sigma-Aldrich®, USA) may be carefully aspirated and eliminated. If trypsin activity had started to remove cells from the substrate, a new growth medium containing fetal bovine serum was introduced.

Phenotype Characterization MSCs by Flowcytometry

After co-culture, MSCs were extracted by centrifugation and converted into single cells before flow cytometry examination. After that, the

cells are treated with unlabeled or fluorescently-conjugated antibodies in test tubes or microtiter plates and evaluated using flow cytometry (Becton Dickson FACSVerse, San Diego, USA). After trypsin was added to identify various MSCs surface markers, 2 x10^5 cells per sample were twice washed in PBS. Anti-CD34 Allophycocyanin (Cat. No. I345804) and anti-CD105 Fluorescein Isothiocyanate (FITC) (Cat. No. 561443) were the antibodies for surface markers (Becton Dickson Pharmingen, San Diego, USA).

This study involved *in vitro* exploratory laboratory experiments on stem cell culture. Following the ethical codes of conduct for research, BMMSCs were removed and extracted from the femur of male Wistar rats weighing 250 grams, who were 4 months old. This was done at the Faculty of Dental Medicine, Universitas Airlangga. In a culture dish measuring 10 cm^2, the cells were plated. The culture was kept alive at 37°C in a humid atmosphere with 5% CO^2. The medium was changed and the non-adherent cells were taken out after 48 hours.

Adherent cells were trypsinized with 0.02% trypsin (Hyclone, Logan, UT) at 37° C for 5 min. when 80–90% confluence was reached. MSCs were cultivated in 24 well plates with 10% fetal bovine serum, penicillin-streptomycin sulfate, and Modified Eagle Medium (MEM). Thereafter, for 5 days, mangosteen peel extract (5 g/ml) was applied to the cell culture. In this investigation, there were two groups of MSC cultures: one with additional mangosteen peel extract and the other without. the use of ELISA for SDF-1 and CXCR4 expression analysis and immunocytochemistry for VEGF expression analysis.

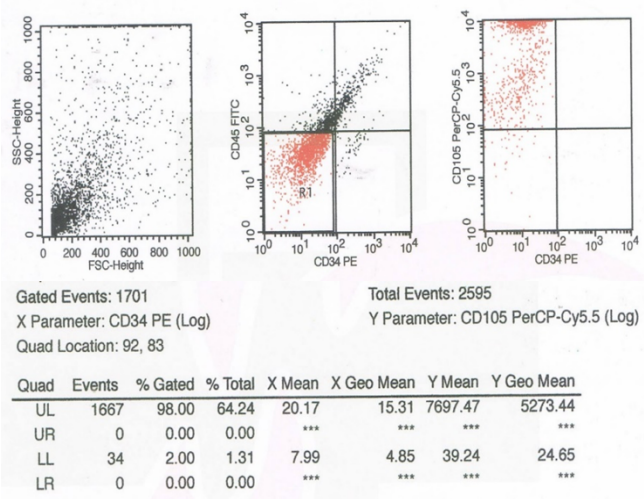
Identification of CXCR4 and SDF-1 by using enzyme linked immunosorbent assay (ELISA)

The MSCs growth media was taken out, and the cells were then lysed in 0.02% sodium dodecyl sulfate in DNAse-free water. The PicoGreen dsDNA Quantitation Kit was then used to count the number of cells in the sample. Excitation at 485 nm and emission at 535 nm was used to measure fluorescence. The CXCR4 and SDF-1 ELISA kit was used to examine the material that had been collected.

Results

This research was conducted to determine the effect of mangosteen (*G. Mangostana L)* peel extract on the expression of some chemokines and growth factors that play a role in mobilizing endogenous mesenchymal stem cells to the injury site. The expression of SDF-1, CXCR4, and VEGF as migration factors in MSCs culture were compared between the groups of MSCs with added mangosteen (*G. Mangostana L)* peel extract and the group of MSCs without mangosteen (*G. Mangostana L)* peel extract induction. Previously, phenotype characterization was done by identifying the protein markers CD105 and –CD34 as positive and negative markers of MSCs by flow cytometric examination. Meanwhile, the protein markers CD90 and CD45 which are positive markers and negative markers of MSCs were identified using immunofluorescence. This aims to ensure the validity that the cultured stem cells are mesenchymal stem cells. Overall research results can be seen as follows:

Characterization of the CD105 and CD34 phenotypes of MSCs by Flowcytometry.



Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	1667	98.00	64.24	20.17	15.31	7697.47	5273.44
UR	0	0.00	0.00	***	***	***	***
LL	34	2.00	1.31	7.99	4.85	39.24	24.65
LR	0	0.00	0.00	***	***	***	***

Figure 1. Examination of the MSCs CD105 phenotype by flowcytometry.

Characterization of the CD90 and CD45 phenotypes of MSCs by Immunofourecence.

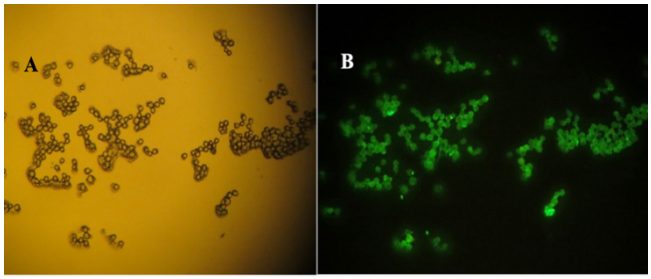


Figure 2. The results of the MSCs CD 90 phenotype examination (A) Without a filter (B)

Using a filter

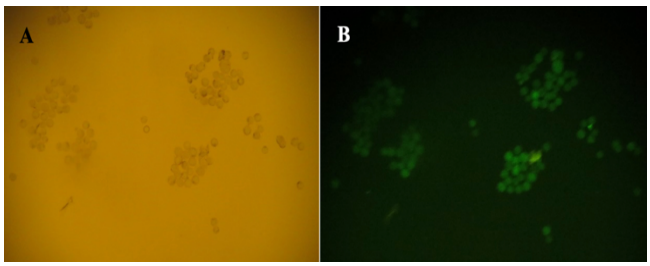


Figure 3. Examination of the CD45 MSCs phenotype (A) Without a filter (B) Using a filter.

Examination of SDF-1 and CXCR4 by ELISA method.

	N	Mean	SD	p
Group 1	20	11.946	1.762	<0.001*
Group 2	20	4.18	0.800	

***significant(p<0.05)**

Table 1. Mean SDF-1 values in the BMMSCs cells culture between groups.

	N	Mean	SD	p
Group 1	20	13.142	1.763	<0.001*
Group 2	20	5.05	0.506	

***significant(p<0.05)**

Table 2. Mean CXCR4 values in the MSCs cells culture between groups.

Examination of VEGF expression by immunocytochemistry

	N	Mean	SD	p
Group 1	20	19.6	2.346	<0.001*
Group 2	20	5.9	1.683	

***significant(p<0.05)**

Table 3. Mean VEGF values in the MSCs cells culture between groups.

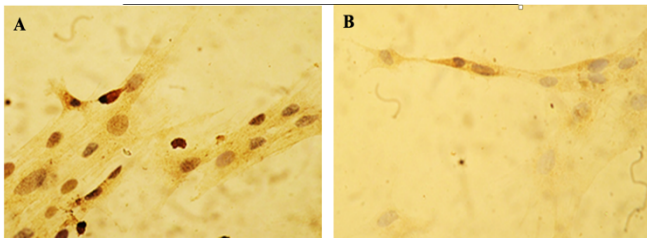


Figure 4. Immunocytochemical examination results showed a significant increase in VEGF expression on MSCs culture with mangosteen peel extract (A) compared with without extract (B).

Figure 1 showed a positive expression (64.24%) and a negative expression CD34 (1.31%), Figure 2 showed a showed a strong positive expression (green luminescence) in the MSCs culture. Figure 3 showed negative expression (no fluorescent) in the MSCs culture. The results of examinations on CXCR4 and SDF-1 performed by ELISA showed a significant increase ($p<0.05$) in expression both on CXCR4 and SDF-1 in MSCs cultures that had been induced by mangosteen peel extract compared to MSCs cultures without mangosteen peel extract (Table 1 and 2). Meanwhile, the results of the examination of VEFG using the immunocytochemical method also showed a significant increase ($p<0.05$) in VEGF expression in MSCs culture induced by mangosteen peel extract compared to MSCs culture without mangosteen peel extract (Table 3 and Figure 4)

Discussion

BMMSCs with all their properties and potencies are a potential source for cell-based regenerative therapy. Currently, the main concern of stem cell therapy is more focused on stem cell transplantation. Weak engraftment and lack of ability to survive when stem cells are transplanted become the main drawbacks of this therapy.[12] So it is necessary to do a new method to optimize the potential of endogenous stem cells in the cell regeneration process. In this study, research has been done using mangosteen (*G. Mangostana L)* peel extract as a natural ingredient to induce endogenous stem cell mobilization to the injured area. In the early stages, research was done on stem cell cultures where 5 µg / ml mangosteen (*G. Mangostana L)* peel extract was added to the culture cells to determine whether the mangosteen (*G. Mangostana L)* peel extract was able to induce several proteins, chemokines, and growth factors, such as VEGF, CXCR4 and SDF-1. These factors have an important role in mobilizing endogenous stem cells to parts of the body that have been injured and require repair and regeneration.[13] The result proves that mangosteen (*G. Mangostana L)* peel extract which is known to contain xanthones as the main element and has a high anti-inflammatory and antioxidant effect

can induce the expression of some proteins, growth factors, and chemokines, including VEGF, CXCR4, and SDF-1 which have an important role in mobilizes endogenous stem cells to the injured area.[14]

Xanthone possesses an anti-oxidant property that includes giving hydrogen atoms from hydroxyl groups to unpaired electrons of reactive oxygen species, according to the chemical makeup of α-mangostin (ROS).[15] Several xanthones produced from mangosteen (*G. Mangostana L)*, including gartanin, γ-mangostin, smeathxanthone A, and α-mangostin, have been demonstrated to have considerable antioxidant activity.[16] ROS are important for angiogenesis. Prior research has demonstrated that hypoxia and growth factors cause the formation of ROS and up-regulate the levels of VEGF and VEGFR. Moreover, ROS stimulates the expression of VEGF and boosts the DNA binding activity of the hypoxia-inducible factor 1α (HIF-1α). Moreover, during angiogenesis, VEGF signaling uses ROS as the second messenger intermediates downstream of VEGFR2.[17]

The following experiment will use Wistar rats subjected to high radiation doses in the ventral region of the rat's neck to receive oral administration of mangosteen (*G. Mangostana L)* peel extract. In this study, it is believed that using mangosteen (*G. Mangostana L)* extract can both protect the affected area and help to repair salivary gland damage brought on by the effects of radiation therapy on the head and neck region. Supplying mangosteen (*G. Mangostana L)* peel extract should be able to activate and direct endogenous stem cells to the location of the injury. Consequently, the difficulty of injecting stem cells into the body in the future can be eliminated, particularly in situations of salivary gland impairment brought on by radiation therapy side effects.

Conclusions

The findings of this study demonstrated that mangosteen (*G. mangostana. L*) peel extract can increase the production of several proteins, growth factors, and chemokines, including VEGF, CXCR4, and SDF-1, which are crucial for attracting endogenous stem cells to the site of injury.

Declaration of Interest

The authors report no conflict of interest.

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References

1. Narmada IB, Laksono V, Nugraha AP, Ernawati DS, Winias S, Prahasanti C, Dinaryanti A, Susilowati H, Hendrianto E, Ihsan IS, Rantam FA. Regeneration of Salivary Gland Defects of Diabetic Wistar Rats Post Human Dental Pulp Stem Cells Intraglandular Transplantation on Acinar Cell Vacuolization and Interleukin-10 Serum Level. Pesquisa Brasileira em Odontopediatria e Clínica Integrada 2019; 19(e5002):1-10.
2. Suciadi SP, Nugraha AP, Ernawati DS, Ayuningtyas NF, Narmada IB, Prahasanti C, Dinaryanti A, Ihsan IS, Hendrianto E, Susilowati H, Rantam FA. The Efficacy of Human Dental Pulp Stem Cells in regenerating Submandibular Gland Defects in Diabetic Wistar Rats (Rattus novergicus). Research J. Pharm. and Tech. 2019; 12(4): 1573-1579.
3. Chin Y, Kinghorn AD. Structural characterization, biological effects, and synthetic studies on xanthones from mangosteen (Garcinia mangostana), a popular botanical dietary supplement. Mini Rev. Org. Chem. 2008;5:355–364.
4. Carpenter HG and Cotroneo E. Salivary Gland Regeneration. In Tucker AS and Miletich I. Salivary Gland : Development, Adaptations and Disease. Renhardt Druck Publisher. 2010. Vol 14 : pp 107-128.
5. Coppess RP and Stokman MA. Stem cells and the repair of radiation-induced salivary gland damage. J.Oral Disease. 2011 March; 17(2) : 143-153.
6. Amano H, Kato S, Ito Y, et al. The Role of Vascular Endothelial Growth Factor Receptor-1 Signaling in the Recovery from Ischemia. PLoS One. 2015;10(7):e0131445.
7. Fukuda S, Bian H, King AG, Pelus LM. The chemokine GRObeta mobilizes early hematopoietic stem cells characterized by enhanced homing and engraftment. Blood 2007; 110(3): 860-9.
8. Luo Q, Zhang B, Kuang D, Song G. Role of Stromal-Derived Factor-1 in Mesenchymal Stem Cell Paracrine-Mediated Tissue Repair. Curr Stem Cell Res Ther 2016;11(7):585-592.
9. Fox A, Smythe J, Fisher N, Tyler MP, McGrouther DA, Watt SM, Harris AL. Mobilization of endothelial progenitor cells into the circulation in burned patients. Br J Surg 2008; 95(2): 244-51
10. Do HTT, Cho J. Mangosteen Pericarp and Its Bioactive Xanthones: Potential Therapeutic Value in Alzheimer's Disease, Parkinson's Disease, and Depression with Pharmacokinetic and Safety Profiles. Int J Mol Sci. 2020;21(17):6211
11. Saputra D, Astuti ER, Daraninggar A, Alam AR, Satria FA, Aulia M. The Effect Of Mangosteen Perıcarp (Garcınıa Mangostana L.) Extract Mucoadhesıve Gıngıval Patch On The Mda Levels And The Number Of Mıcronucleı Due To Panoramıc Radıography Radıatıon. Biochemical and Cellular Archives 2020; 20(2): 4877–4882
12. Rantam FA, Nugraha AP, Ferdiansyah F, Purwati P, Bumi C, Susilowati H, Hendrianto E, utomo DN, Suroto H, Sumartono C, Setiawati R, Prakoeswa CR, Indramaya DM. A Potential Differentiation of Adipose and Hair Follicle-derived Mesenchymal Stem Cells to Generate Neurons Induced with EGF, FGF, PDGF and Forskolin. Research J. Pharm. and Tech. 2020; 13(1): 275-281.
13. Melincovici CS, Boşca AB, Şuşman S, et al. Vascular endothelial growth factor (VEGF) - key factor in normal and pathological angiogenesis. Rom J Morphol Embryol. 2018;59(2):455-467.
14. Mulyani SWM, Ernawati DS, Astuti ER, Rantam FA. Hypoxic preconditioning effect on stromal cells derived factor-1 and C-X-C chemokine receptor type 4 expression in Wistar rat's (Rattus norvegicus) bone marrow mesenchymal stem cells (in vitro study), Veterinary World 2018;11(7): 965-970.
15. Ovalle-Magallanes B, Eugenio-Pérez D, Pedraza-Chaverri J. Medicinal Properties of Mangosteen (Garcinia mangostana L.): A Comprehensive Update. Food Chem Toxicol. 2017; 109: 102-122.
16. Jung HA, Su BN, Keller WJ, Mehta RG, Kinghorn AD. Antioxidant xanthones from the pericarp of Garcinia mangostana (Mangosteen). J Agric Food Chem 2006; 54: 2077–2082.
17. Deudero JJ, Caramelo C, Castellanos MC, Neria F, Fernandez-Sanchez R, Calabia O, Penate S, Gonzalez-Pacheco FR. Induction of hypoxia-inducible factor 1α gene expression by vascular endothelial growth factor. J Biol Chem. 2008;283:11435–11444.