Dear Wahyu Widowati,

Your submission "Induction of Cartilage Degradation by Interleukin IL-1β as Osteoarthritis Model" (MSID:8428 which part of ICAMBBE 2017) to Journal of Mathematical and Fundamental Sciences now needs to be proofread by following these steps.
1. Download and review thoroughly the attached file.
2. Enter corrections (typographical and format) in Proofreading Corrections, the format for the correction is available below the signature. You have to highlight the manuscript with yellow highlight tools to indicate the word(s) or phrase or equation that should be fixed.
3. Save the notes and send email corrections to the publication assistant (this email) as well as the marked PDF file. (Please change the end of the file name from "LE" to "AuthLE" to indicate the file has been changed by Authors).

Please note that due date for Author Proofreading will be February 5th, 2019.

Submission URL:
http://journals.itb.ac.id/index.php/jmfs/author/submissionEditing/8428
Username: wahyu_w60

Thank you.

Kind regards.

Budhi Mulyawan
Journal of Mathematical and Fundamental Sciences ITB Journal Publisher Institut Teknologi Bandung jmfs@lppm.itb.ac.id

-----Original Message-----
From: Journal of Mathematical and Fundamental Sciences [mailto:jmfs@lppm.itb.ac.id]
Sent: 29 January 2019 16:31
To: Wahyu Widowati
Cc: Committee ICAMBBE; jmfsitb@gmail.com
Subject: [jmfs] Proofreading Request (Author) (MSID:8428)

Dear Wahyu Widowati,
Your submission "Induction of Cartilage Degradation by Interleukin IL-1β as Osteoarthritis Model" (MSID:8428 which part of ICAMBBE 2017) to Journal of Mathematical and Fundamental Sciences now needs to be proofread by following these steps.
1. Download and review thoroughly the attached file.
2. Enter corrections (typographical and format) in Proofreading Corrections, the format for the correction is available below the signature. You have to highlight the manuscript with yellow highlight tools to indicate the word(s) or phrase or equation that should be fixed.
3. Save the notes and send email corrections to the publication assistant (this email) as well as the marked PDF file. (Please change the end of the file name from "LE" to "AuthLE" to indicate the file has been changed by Authors).
Please note that due date for Author Proofreading will be February 5t, 2019.
Submission URL:
http://journals.itb.ac.id/index.php/jmfs/author/submissionEditing/8428
Username: wahyu_w60

Thank you.
Kind regards.

Budhi Mulyawan
Journal of Mathematical and Fundamental Sciences ITB Journal Publisher Institut Teknologi Bandung jmfs@lppm.itb.ac.id

==

===

Proofing Instructions
The proofreading stage is intended to catch any errors in the galley's spelling, grammar, and formatting. More substantial changes cannot be made at this stage unless discussed with the Section Editor. In Layout, click on VIEW PROOF to see the HTML, PDF, and other available file formats used in publishing this item.
For Spelling and Grammar Errors
Copy the problem word or groups of words and paste them into the Proofreading Corrections box with "CHANGE-TO" instructions to the editor as follows (below are examples):

1. CHANGE...
   then the others
   TO...
   than the others

2. CHANGE...
   Malinowsky
   TO...
   Malinowski

For Formatting Errors
Describe the location and nature of the problem in the Proofreading Corrections box after typing in the title "FORMATTING" as follows (below are
examples):

3. FORMATTING
   The numbers in Table 3 are not aligned in the third column.

4. FORMATTING
   The paragraph that begins "This last topic..." is not indented.

Induction of Matrix Metalloproteinases in Chondrocytes by Interleukin IL1β as an Osteoarthritis Model

Ervi Afifah[1], Tjandrawati Mozef[2], Ferry Sandra[3], Seila Arumwardana[1], Dwi Davidson Rihibiha[1], Hayatun Nufus[1], Rizal Rizal[1], Annisa Amalia[1], Indra Bachtiar[4], Harry Murti[4] & Wahyu Widowati[5]

[1]Biomolecular and Biomedical Research Center, Aretha Medika Utama, Bandung 40163, Jawa Barat, Indonesia
[2]Research Center for Chemistry, Indonesian Institute of Sciences, Serpong, Indonesia
[3]Department of Biochemistry and Molecular Biology, Faculty of Dentistry, Trisakti University, Jakarta, Indonesia
[4]Stem Cell and Cancer Institute, Jakarta 13210, Indonesia
[5]Medical Research Center, Faculty of Medicine, Maranatha Christian University, Bandung 40164, Jawa Barat, Indonesia
E-mail: wahyu_w60@yahoo.com

Abstract. Osteoarthritis (OA) is a chronic disease of the joints and bones due to trauma or other joint-related diseases (secondary). Synovial inflammation commonly causes disturbance in joint homeostasis, which is associated with OA. Enzymes such as aggrecanase and metalloproteinase generate cartilage damage, mediated by tumor necrosis factor (TNF) and interleukin (IL)-1. Pro-inflammatory cytokines, including TNF-α, IL1β, and IL-6, are responsible for regulation of the extracellular matrix, cartilage degradation, and apoptosis of chondrocytes. This study aimed to observe the cell viability and expression level of matrix metalloproteinases (MMP1 and MMP3) and tissue inhibitor metalloproteinases (TIMP1 and TIMP2) in human chondrocyte cells (CHON002) induced by IL1β. CHON002 was induced with IL1β (0.1, 1 and 10 ng/mL) as an OA model. The viability of the cells was measured with a 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxyme-thoxyphenyl)-2-(4-sulfophenyl)-2H-tetra zolium (MTS) assay, while expression of MMP1, MMP3, TIMP1, and TIMP2, was evaluated by RT-PCR. The viability of IL1β-induced CHON002 (CHON002- IL1β) cells at day 1 and 5 showed that treatment with up to 10 ng/mL of IL1β was not toxic. Expression of TIMP1 and TIMP2 in CHON-IL1β was lower compared to control, while that of MMP1 and MMP3 was higher compared to control. These results indicate that CHON002 treated with 10 ng/mL IL1β has expression patterns consistent with chondrocyte damage, so the CHON-IL1β system may serve as a model for MMP induction in OA.

Keywords: *interleukin; mesenchymal stem cell; metalloproteases; osteoarthritis; MMP genes.*

Received September 25th, 2017, Revised November 16th, 2017, Accepted for publication July 25th 2018.
 ISSN: 2337-5760, DOI: 10.5614/j.math.fund.sci.2019.xx.x.x

1 Introduction

The Osteoarthritis (OA) is a chronic disease of the joints and bones due to trauma or other joint-related diseases [1]. OA is also associated with synovial inflammation, which causes disturbance of joint homeostasis [2]. Until now, an effective pharmacotherapy that can repair the function and the original structure of the damaged articular cartilage has not been found. Current therapies for OA use anti-inflammatory medication such as nonsteroidal anti-inflammatory drugs (NSAIDs). Biological therapies for OA and other orthopedic disorders are still being investigated [3]. Mesenchymal stem cells (MSCs) have been selected for OA therapy, because they are able to differentiate into chondrocytes and have the ability to repair articular cartilage. MSCs can be isolated from various types of mature tissue without reducing the potency of differentiation [3]. The aim of this research was to find an osteoarthritis cell line model that can be used on treatment with MSCs in OA therapy.

In this research, human chondrocyte cells (CHON002) were treated with IL1β as a model of OA. IL1β also promotes OA occurrence through several mechanisms: imbalance between damage and recovery of cartilage, reactive oxygen species (ROS), including nitrite oxide (NO), and inflammatory mediators such as prostaglandin E2 (PGE2) through increasing cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) [4]. Free radicals induce oxidative stress, which can lead to an imbalance in both pro-oxidants and antioxidants, which further results in joint damage in patients with OA and rheumatoid arthritis (RA) [5].

Matrix metalloproteinases (MMPs) play a major role in the processes of chondrolysis. They are responsible for degenerative changes in the OA cartilage [6,7]. The messenger RNA (mRNA) identified in human cartilage, based on recent studies, comprises MMP1, MMP3, MMP9, and MMP13 [8,9], type II collagen degradation products by collagenase and also specific MMP proteins [10-12]. This type of enzyme is related to an intrinsic chondrocyte-mediated degenerative change of the cartilage matrix in OA. However, the importance of these enzymes remains unclear. The activities of metalloproteinase can be inhibited by an endogenous metalloproteinase inhibitor, i.e. tissue inhibitors of metalloproteinases (TIMPs) [13]. The complex regulation of MMP production by chrondrocytes is affected by several factors, one of which is their response to pro-is cytokines, including IL1 or TNF [14-18]. This study was aimed at observing the levels of MMP1 and MMP3 and their inhibitors, TIMP1 and TIMP2, in CHON002 induced by IL1β.

2 Materials and Methods

2.1 Cell Culture

Human long bone cartilage (CHON002 ATCC ® CRL-2847™) was obtained from Aretha Medika Utama, Biomolecular and Biomedical Research Center, Bandung, Indonesia. The cells were cultured and maintained in Dulbecco's Modified Eagle Media (DMEM, Gibco 11995065) supplemented with 10% (v/v) fetal bovine serum (FBS, Gibco 10270106), 1% anti-biotic and anti-mycotic (Gibco, 152400620) and 1% geneticin (Gibco, 10151027), and incubated in a humidified atmosphere at a temperature of 37 ºC in 5% CO2 [18].

2.2 Induction with IL1β

CHON002 cells (10^6 cells) were seeded into T-25 flasks and incubated for 2-3 days. Based on a cytotoxicity assay, the viability results for various concentrations showed that the IL1β was not toxic. Thus, a concentration between 5 and 10 ng/mL is safe and was used for the treatment in this study. The medium was replaced with media containing 5 or 10 ng/mL recombinant IL1β (Biolegend, 579404). The cells were induced for 5 days [18].

2.3 Cytotoxicity Testing for Viability of CHON002 Cells after IL1β Induction

CHON002 cells at 80% confluency were washed with 1 mL of 1X phosphate buffered saline (PBS, Gibco 14200075) twice. The cells and 1 mL of Trypsin-EDTA (0.25%) were added to phenol red (Gibco, 25200072) and then incubated at 37 °C in 5% CO2 for 10 min to remove attached cells. One mL of complete medium was then added to stop trypsin digestion. The suspension was centrifuged for 5 min at 1500 rpm. The supernatant was removed and the pellet was homogenized with 1 mL of complete medium. The cells were counted with a haemacytometer after 10x dilution. The cells were plated at 5000 cells/well for 100 µL medium. The cells were added into a 96-well plate and then incubated in 5% CO¬2 at 37 °C for 24 hours. Medium was removed and added with 90% fresh complete medium and 10% of IL1β. The cells were incubated at 37 °C in 5% CO¬2 for 24 hours, each well was added with 20 µL of 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxyme-thoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) (Abcam, ab197010), and the plate was incubated in 5% CO¬2 for 3 hours at 37 °C. Absorbance was measured at 490 nm wavelength using a microplate reader (Thermo Scientific Multiskan Go) [19].

2.4 RNA Extraction and cDNA Synthesis

RNA extraction was done using an Aurum Total RNA Kit (Bio-Rad, 7326820) based on the manufacturer's instructions. Afterwards, the purity of the RNA was measured by spectrophotometry at 260/280 nm with a Thermo Scientific Multiskan Go. Then, the obtained RNA was used for cDNA synthesis using an iScript cDNA synthesis kit (Bio-Rad, 1708890) for 5 min at 25 °C, 30 min at 42 °C, and in the last step for 5 min at a temperature of 85 °C. The product obtained was stored at -20 °C [20].

2.5 mRNA Level Quantification of TIMP1, TIMP2, MMP1, and MMP3 Genes

Table 1 shows the primer used in this study. Real-time qPCR was done using a PikoReal Real-time PCR System (Thermo Scientific Inc.) with pre-incubation for 5 min at a temperature of 95 °C, continued with 40 cycles of denaturation for 1 min at 95 °C, with the following annealing temperatures: TIMP1 and TIMP2 at 50 °C, MMP1 at 54 °C and MMP3 at 52 °C for 40 sec, and elongation for 1 min at 72 °C. The reaction mix that was used to perform qPCR was Evagreen Master Mix (Bio-Rad, 1725200) [20].

Table 1 Primer Sequences.

Primer	Forward	Reverse
β actin	5'-TCTGGCACCACACCTTCTACAATG-3'	5'-AGCACAGCCTGGATAGCAACG-3'
MMP1	5'-CTGAAGGTGATGAAGCAGCC-3'	5'-AGTCCAAGAGAATGGCCGAG-3'
MMP3	5'-CTCCACGAGCTTGTAGGAAAG-3'	5'-CACGCCTGAAGGAAGAGATG-3'
TIMP1	5'-AGTCAAGACCACCTTATACCA-'3	5'-AGTCAACCAGACCACCTTATACCA-3'
TIMP2	5'-GGAAGTGGACTCTGGAAACGACATT-3'	5'-CTCGATGTCGAGAAACTCCTGCTTG-3'

2.6 Statistical Analysis

The statistical analysis in this study was conducted using SPSS ver. 16.0 with one-way analysis of variance (ANOVA). The analysis was continued with Duncan tests with significancy $p < 0.05$. The data in this study are presented as mean ± SD.

3 Results

3.1 Viability of CHON002-IL1β

The viability of IL1β-induced CHON002 cells (CHON002-IL1β) was measured using an MTS assay. The assay was based on the formation of purple formazan product from yellow tetrazolium [19]. The cell viability values of the treated cells were compared to control. As can be seen in Table 2, CHON002-IL1β on days 1 and 5 showed that the various concentrations of IL1β (0.1, 1 and 10

ng/mL) and the period of incubation (1 and 5 days) were not toxic. The percentage of viable cells that were given IL1β showed over 100% live cells and higher IL1β concentrations showed increased cell viability.

Table 2 Viability of CHON002 Cells treated with Different Concentrations of IL1β and Periods of Incubation.

Concentration of IL1β	Cell viability (%)	
	Day 1 incubation	Day 5 incubation
Control	100 ± 13	100 ± 25[a]
0,1 ng/mL IL1β	101 ± 14	98 ± 8[a]
1 ng/mL IL1β	105 ± 14	107 ± 4[a]
10 ng/mL IL1β	112 ± 15	125 ± 12[b]

Note: Data are presented as mean ± STD from 3 replications. Superscript letters (a & b) show significant differences between treatment (concentration level of IL1β) on day 5 of incubation based on Duncan's post hoc test with significancy $p < 0.05$.

3.2 Characterization of CHON002-IL1β

Degenerative changes in OA cartilage are affected by chondrolytic processes that involve MMPs. In this study, the levels of MMP1 and MMP3 and its inhibitors, TIMP1 and TIMP2, were measured in osteoarthritis-like characteristics of IL1β-induced CHON002 cells. The levels of TIMP1 and TIMP2 in the IL1β-induced cells were lower than in control. Meanwhile, MMP1 and MMP3 were higher in the IL1β-induced cells compared to the control cells, which indicates chondrocyte damage (Figure. 1).

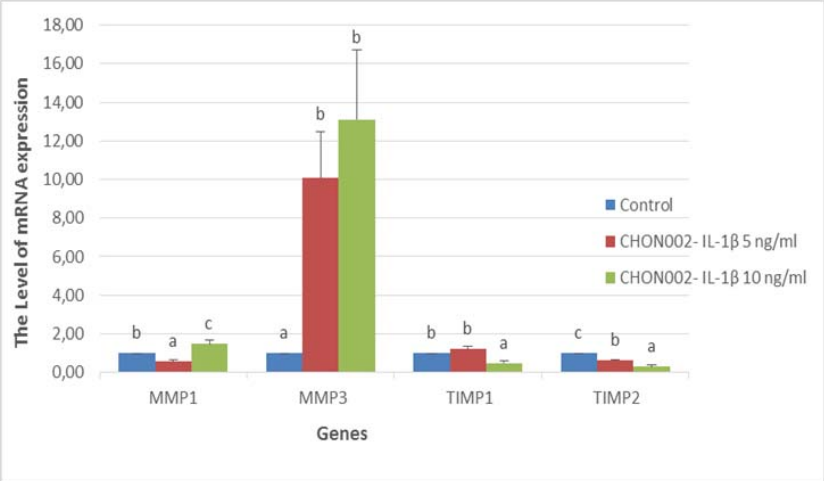

Figure 1 Levels of mRNA expression of MMP1, MMP3, TIMP1 and TIMP2 genes in IL1β-induced CHON002. The data were analyzed with ANOVA and Duncan's post hoc test. The letters (a, b, c) indicate significant differences between treatments ($p < 0.05$) for each parameter (MMPs and TIMPs).

4 Discussion

In this study, IL1β was not toxic to the cell line, as indicated by cell viability above 100% after treatment. This result is not consistent with previous research, where IL1β caused cell death by apoptosis and necrosis. Cell death caused by apoptosis was observed in human chondrocytes in normal OA cartilage after incubation with human IL1β and after ROS-mediated necrosis was suppressed [21].

In the current study, the levels of MMP1 and MMP3 were increased in CHON002-cells treated with 10 ng/mL IL1β, while the levels of its inhibitors, TIMP1 and TIMP3, were decreased. Several previous studies, both in vivo and in vitro, have shown the role of MMPs in cartilage damage [22-24]. Elevated levels of MMP-1 (collagenase-1) and MMP-3 (stromelysin-1) were documented in osteoarthritic cartilage [25,26] and also found in the synovial fluid of osteoarthristic joints [26,27].

TIMPs are known to block collagenolysis effectively, which indicates a role in the inhibition of MMPs in this process, while aggrecanolysis is blocked by TIMP-3 [28], probably by inhibition of ADAMTS-4 and -5 [29].

Cartilage matrix degradation is considered a result of increased activity of chondrocyte-produced enzymes. The cartilage breakdown involves various kinds of cytokines, while OA chondrocytes are able to synthesize IL-6, IL1β, TNF-α, and many other enzymes [9,15]. MMP synthesis has been up-regulated by the proinflamatory cytokines TNF and IL1 by chondrocytes under normal and pathologic conditions [9,18]. Several studies have reported measurable levels of cytokines, such as IL1β and TNF-α, in synovial fluids found in normal and diseased joints, contributing to expression of MMP by chondrocytes [30]. Referring to previous studies, IL1 and TNF can be produced by human chondrocytes in situ and synthesis of these cytokines by local chondrocytes can be an alternative source of MMP induction in OA cartilage [31].

5 Conclusion

Although apoptotic and necrotic mechanisms can cause cell death, up to 10 ng/mL IL1β is not toxic to CHON002 cells. Since this level of IL1 β increased MMP1 and MMP3 expression and decreased TIMP1 and TIMP2 expression, CHON002-IL1β may be a useful model for this aspect of OA.

Acknowledgements

We gratefully acknowledge the financial support of Program Insinas Individu Pratama from the Directorate of Research Empowerment and Development, Ministry of Research, Technology and Higher Education of the Republic of Indonesia (25/INS/PPK/E/E4/2017). We are thankful to Yukko Arinta, Hanna Sari W Kusuma from Aretha Medika Utama, Biomolecular and Biomedical Research Center, Bandung for their valuable assistance. This study was supported by Aretha Medika Utama, Biomolecular and Biomedical Research Center, Bandung, Indonesia by providing the research laboratory, facilities, and methods.

References

[1] Ahmed, U., Anwar, A., Savage, R.S. & Costa, M.L., *Biomarkers of Early Stage Osteoarthritis, Rheumatoid Arthritis and Musculoskeletal Health*, Sci. Reports, pp. 1-7, 2015.

[2] Courties, A., Gualillo, O., Berenbaum, F. & Sellam, J., *Metabolic Stress-Induced Joint Inflammation and Osteoarthritis*, OARS, **23**(11), pp. 1955-1965, 2015.

[3] Noth, U., Steinert, A.F. & Tuan, R.S., *Technology Insight: Adult Mesenchymal Stem Cells for Osteoarthritis Therapy*, Nat. Clin. Pract. Rheumatol., **4**(7), pp. 371-380, 2008.

[4] Cheng, A.W.M., Satbler, T.V., Bolognesi, M. & Kraus, V.B., *Selenomethionine Inhibits IL1β Inducible Nitric Oxide Synthase (iNOS) and Cyclooxygenase 2 (COX2) Expression in Primary Human Chondrocytes*, OARS, **19**(1), pp. 118-125, 2011.

[5] Ozkan, Y., Yardym-Akaydyn, S. & Sepici, A., *Oxidative Status in Rheumatoid Arthritis*, Clin. Rheumatol., **26**(1), pp. 64-68, 2007.

[6] Lu, H., Jiang, J., Xie, G., Liu, W. & Yan, G., *Effects of an Aqueous Extract of Eucommia on Articular Cartilage in a Rat Model of Osteoarthritis of the Knee*, Exp. Ther. Med., **6**(3), pp. 684-688, 2013.

[7] Sokolove, J. & Lepus, C.M., *Role of Inflammation in the Pathogenesis of Osteoarthritis: Latest Findings and Interpretations*, Ther. Adv. Musculoskelet, **5**(2), pp. 77-94, 2013.

[8] Rose, B.J. & Kooyman, D.L., *A Tale of Two Joints: The Role of Matrix Metalloproteases in Cartilage Biology*, Dis. Markers, **2016** , pp. 4895050, 2016.

[9] Higashiyama, R., Miyaki, S., Yamashita, S., Yoshitaka, T., Lindman , G., Ito, Y., Sasho, T., Takahashi, K., Lotz, M. & Asahara, H., *Correlation Between MMP-13 and HDAC7 Expression in Human Knee Osteoarthritis*, Mod. Rheumatol., **20**(1), pp. 11, 2010.

[10] Francis, M.S., Yates, M.P., Vickery, L.E., Klover, J.A., Song, L.R.H., Anglin, C.P. & Misko, T.P., *Protein Geranylgeranylation Controls Collagenase Expression in Osteoarthritic Cartilage*, OARS, **18**(2010), pp. 948–955, 2010.

[11] Tchetian, E.V., Kobayashi, M., Yasuda, T., Meijers, T., Pidoux, I. & Poole, A.R., *Chondrocyte Hypertrophy can be Induced by a Cryptic Sequence of Type II Collagen and is Accompanied by the Induction of MMP-13 and Collagenase Activity: Implications for Development and Arthritis*, Matrix Biol., **26**(2007), pp. 247-258, 2007.

[12] Dejica, V.M., Mort, J.S., Laverty, S., Antoniou, J., Zukor, D.J., Tanzer, M. & Poole, A.R., *Increased Type II Collagen Cleavage by Cathepsin K and Collagenase Activities with Aging and Osteoarthritis in Human Articular Cartilage*, Arthritis Res. Ther., **14**, pp. 113, 2012.

[13] Brew, K. & Nagase, H., *The Tissue Inhibitors of Metalloproteinases (Timps): An Ancient Family with Structural and Functional Diversity*, Biochim. Biophys. Acta., **1803**(1), pp. 55-71, 2010.

[14] Goldring, M.B. & Otero, M., *Inflammation in Osteoarthritis*, Curr. Opin. Rheumatol., **23**(5), pp. 471-478, 2011.

[15] Kapoor, M., Martel-Pelletier, J., Lajeunesse, D., Pelletier, J.P. & Fahmi, H., *Role of Proinflammatory Cytokines in the Pathophysiology of Osteoarthritis*, Nat. Rev. Rheumatol., **7**, pp. 33-42, 2011.

[16] Sellam, J. & Berenbaum, F., *The Role of Synovitis in Pathophysiology and Clinical Symptoms of Osteoarthritis*, Nat. Rev. Rheumatol., **6**, pp. 625-635, 2010.

[17] Haseeb, A. & Haqqi, T.M., *Immunopathogenesis of Osteoarthritis*, Clin. Immunol., **146**, pp. 185-196, 2013.

[18] Tsuchiyaa, K., Chenb, B., Ushidac, T., Matsunoa, T. & Tateishib., T., *The Effect of Coculture of Chondrocytes with Mesenchymal Stem Cells on Their Cartilaginous Phenotype In Vitro*, Mater. Sci., **24**(3), pp. 391-396, 2014.

[19] Widowati, W., Laura, W., Wargasetia, T.L., Bachtiar, I., Yellianty, Yellianty., Laksmitawati & Dian R., *Antioxidant, Anticancer, and Apoptosis-Inducing Effects of Piper Extracts in Hela Cells*, J. Exp. Integr. Med., **3**(3), pp. 225-230, 2013.

[20] Hidayat, M., Prahastuti, S., Fauziah, N., Maesaroh, Balqis. & Widowati, W., *Modulation of Adipogenesis Related Gene Expression by Ethanol Extracts of Detam 1 Soybean and Jati Belanda Leaf in 3T3-L1 Cells*, Banglaesh, J. Parmaco., **11**, pp. 697-702, 2016.

[21] Daheshia, M. & Yao, J.Q., *The Interleukin 1ß Pathway in the Pathogenesis of Osteoarthritis*, J. Rheumatol., **35**(12), pp. 2306-2312, 2015.

[22] Blom, A.B., van Lent, P.L., Libregts, S., Holthuysen, A.E., van der Kraan, P.M., van Rooijen, N. & van den Berg, W.B., *Crucial Role of*

Macrophages in Matrix Metalloproteinase-mediated Cartilage Destruction during Experimental Osteoarthritis: Involvement of Matrix Metalloproteinase 3, Arthritis. Rheum., **56**(1), pp. 147-157, 2007.

[23] Goldring, M.B., Otero, M., Plumb, D.A., Dragomir, C., Favero, M., Hachim, K.E., Hashimoto, K., Roach, H.I., Olivotto, E., Borzi, R.M. & Marcu, K.B., *Roles of Inflammatory and Anabolic Cytokines in Cartilage Metabolism: Signals and Multiple Effectors Converge Upon MMP-13 Regulation in Osteoarthritis*, Eur. Cell. Mater., **21**, pp. 202-220, 2011.

[24] Little, C.B., Abarai, A., Burkhardt, D., Smith, S.M., Fosang, A.J., Werb, Z., Shah, M. & Thompson, E.W., *Matrix Metalloproteinase 13–Deficient Mice Are Resistant to Osteoarthritic Cartilage Erosion but Not Chondrocyte Hypertrophy or Osteophyte Development*, Arthritis. Rheum., **60**(12), pp. 3723-3733, 2009.

[25] Hulejova, H., Baresova, V., Klezl, Z., Polanska, M., Adam, M. & Senolt L., *Increased Level of Cytokines and Matrix Metalloproteinases in Osteoarthritic Subchondral Bone*, Cytokine, **38**(2007), pp. 151-156, 2007.

[26] Koskinen, A., Vuolteenaho, K., Nieminen, R., Moilanen, T. & Moilanen, E., *Leptin Enhances MMP-1, MMP-3 and MMP-13 Production in Human Osteoarthritic Cartilage and Correlates with MMP-1 and MMP-3 in Synovial Fluid from OA Patients*, Clin. Exp. Rheumatol., **29**, pp. 57-64, 2011.

[27] Scanzello, C.R., Umoh, E., Pessler, F., Diaz-Torne, C., Miles, T., DiCarlo, E., Potter, H.G., Mandl, L., Marx, R., Rodeo, S., Goldring, S.R., Crow, M.K., *Local Cytokine Profiles in Knee Osteoarthritis: Elevated Synovial Fluid Interleukin-15 Differentiates Early from End-Stage Disease*, OARS, **17**(8), pp. 1040-1048, 2009.

[28] Rowan, A.D. & Young, D.A., *Collagenase Gene Regulation by Pro-Inflammatory Cytokines in Cartilage*, Front Biosci, **12**, pp. 536-550, 2007.

[29] Gendron, C., Kashiwagi, M., Hughes, C., Caterson, B. & Nagase, H., *TIMP-3 Inhibits Aggrecanase-Mediated Glycosaminoglycan Release from Cartilage Explants Stimulated by Catabolic Factors*, FEBS Lett., **555**, pp. 431-436, 2003.

[30] Kashiwagi, M., Tortorella, M., Nagase, H. & Brew, K., *TIMP-3 is a Potent Inhibitor of Aggrecanase 1 (ADAM-TS4) and Aggrecanase 2 (ADAM-TS5)*, J. Biol. Chem., **276**, pp. 12501-12504, 2001.

[31] Moon, D.O., Kim, M.O., Choi, Y.H., Park, Y.M. & Kim, G.Y., *Curcumin Attenuates Inflammatory Response in IL1β-Induced Human Synovial Fibroblasts and Collagen-Induced Arthritis in Mouse Model*, Int. Immunopharmacol, **10**(5), pp. 605-610, 2010.