

# Justicia gendarussa Ethanolic Extract Enhance Bone Matrix Deposition in in vitro study

Kavita Supparmaniam, Siti Pauliena Mohd Bohari\*

Department of Biotechnology and Medical Engineering, Faculty of Biosciences and Medical Engineering, Universiti Teknologi Malaysia, 81310 Skudai, Johor, Malaysia \*Corresponding author: *pauliena@fbb.utm.my* 

# **ABSTRACT**

Justicia gendarussa (Acanthaceae) or commonly known as Gendarussa has traditionally been used to treat bone fractures. Bone fracture is a clinical condition that need bone repair and new bone formation. To date, the mechanism of Justicia gendarussa acting in enhancing the bone mineralization has not been proven scientifically. The present study aimed to investigate the cytotoxicity and alkaline phosphatase (ALP) activity on osteoblast cells when treated with Justicia gendarussa ethanolic leaves extract. For cell viability, the result showed that IC<sub>50</sub> value of the osteoblast cells was 89.1µg/ml. While, ALP assay is used as a biochemical marker for early detection of osteoblast mineralization. The highest amount of ALP activity was at the 37.5 µg/ml when compared to the control. From this study, it shows that Justicia gendarussa has potential in enhancing bone mineralization during the bone repair process.

## **INTRODUCTION**

Bone fracture is one of the clinical condition that caused either by trauma like accidents and few diseases like osteoporosis, tumor and deficiency of calcium or vitamin D.<sup>1</sup> Although there are number of treatment, the healing of bone fracture usually takes a longer period, ranging from six to eight weeks.<sup>2</sup> Traditional herbal medicines which have been used in medical practice might play important role in bone fracture healing.<sup>1</sup>





Concentration (µg/ml)	Control	7.81	15.63	31.25	62.5	125	250	500	1000
		***	**	***	***	*	***	***	*
% of cell	100	72.71	70.94	64.99	61.29	40.96	26.34	16.02	8.68
viability	±0.008	±0.001	±0.009	±0.009	±0.001	±0.005	±0.009	±0.318	±0.012

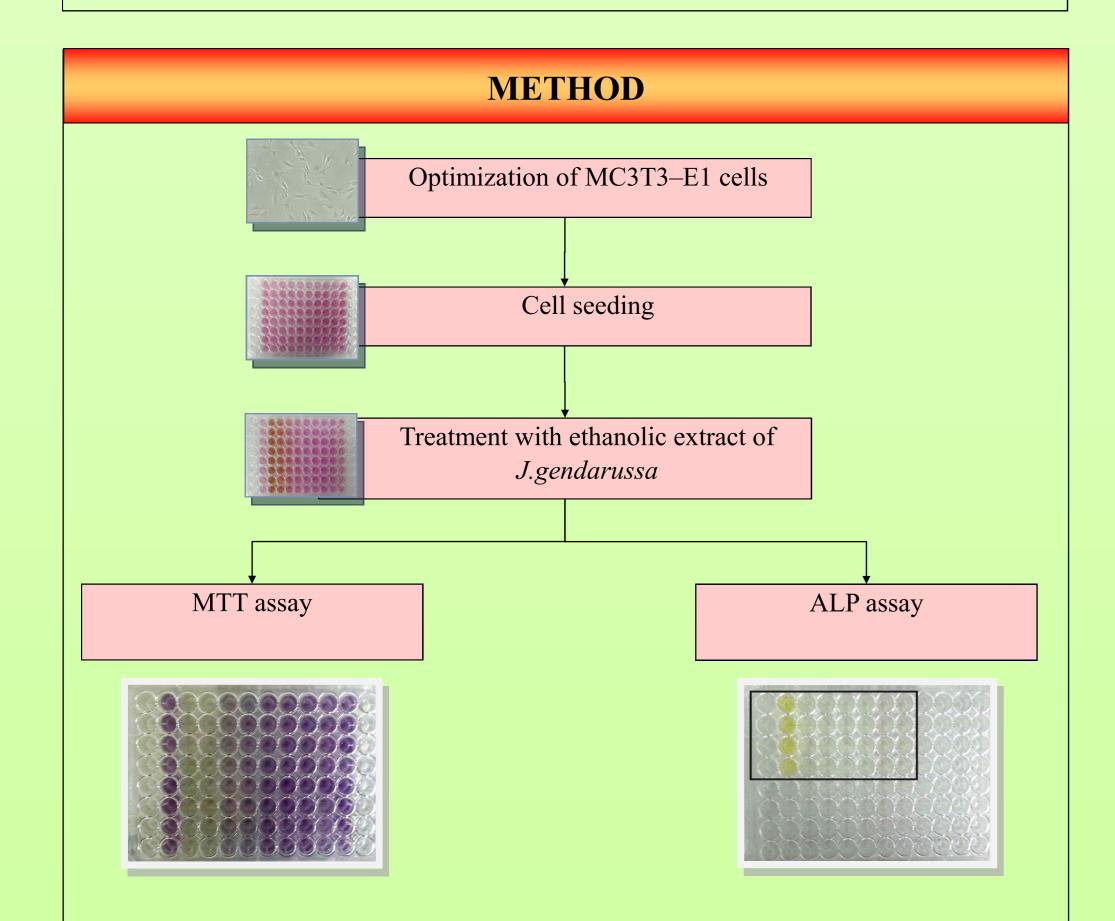
Values are mean ± STDEV for three replicates; \*p<0.05, \*\*p<0.01 and \*\*\*p<0.001 compared with control

Table 1: Percentage of cell viability of MC3T3-E1 cells treated with different concentrations of J.gendarussa

#### ethanolic extract.

# **OBJECTIVE**

To investigate the cytotoxicity and alkaline phosphatase (ALP) activity of osteoblast cells when treated with ethanolic leaves extract of J. gendarussa.



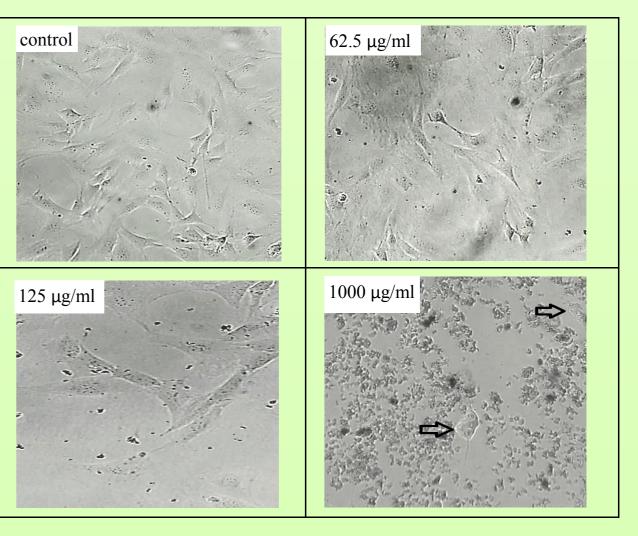


Figure 1: Morphology of MC3T3-E1 cells in MTT assay after 72 hours treated with different concentration of J.gendarussa ethanolic extract (200× magnification).

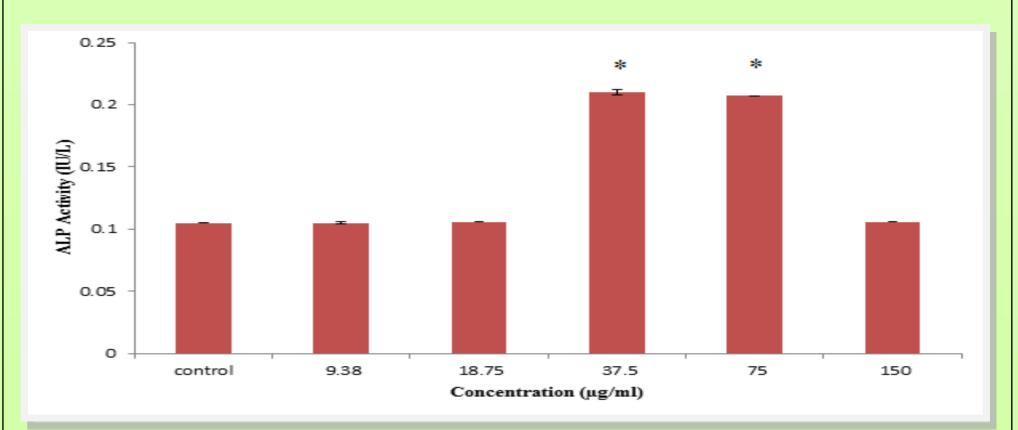


Figure 2: ALP activity of MC3T3-E1 cell treated with different concentrations of J.gendarussa ethanolic extract. Values are expressed as the mean±SD for three replicates, \*p<0.05 compared with control.

#### **REFERENCES**

- Singla, C., Drabu, S., Verma, R., Dhiman, A., & Sharma, A. (2011). Recent update on proficient bone fracture revivifying herbs. IRJP, 2(11), 3-5.
- Singh, V., Singh, N., Pal, U., Dhasmana, S., Mohammad, S., & Singh, N. (2011). Clinical evaluation of cissus quadrangularis and moringa oleifera and osteoseal as osteogenic agents in mandibular fracture. National journal of maxillofacial surgery, 2(2), 132.
- Park, J.-B. (2012). Effects of 17-α ethynyl estradiol on proliferation, differentiation & mineralization of osteoprecursor cells. The Indian journal of medical research, 136(3), 466.
- Sugawara, Y., Suzuki, K., Koshikawa, M., Ando, M., & Iida, J. (2002). Necessity of enzymatic activity of alkaline phosphatase for mineralization of osteoblastic cells. The Japanese journal of pharmacology, 88(3), 262-269.
- Heino, T. J., Hentunen, T. A., & Väänänen, H. K. (2004). Conditioned medium from osteocytes stimulates the proliferation of bone marrow mesenchymal stem cells and their differentiation into osteoblasts. Experimental cell research, 294 (2), 458-468.

#### DISCUSSION

The IC<sub>50</sub> value showed the *J.gendarussa* ethanolic extract is not toxic towards MC3T3-E1 cells. At the highest concentration (1000 µg/ml), morphology of the MC3T3-E1 cells changed into a rounded shape when compared to the control. Based on the result, it is suggested that at lower concentration (below than 100 µg/ml), this plant extract could stimulate the ALP activity in osteoblast cell. ALP is an early marker which used to detect osteoblast cell differentiation and the ALP activity is elevated when there is increased in osteoblast cell differentiation. <sup>3,4</sup> Differentiation of osteoblast cell to become osteocyte is the final phase of differentiation, where the osteocyte cells embedded in the mineralized bone matrix and forms bone.<sup>5</sup> From this, we can suggest that *J.gendarussa* can increase osteoblastic differention into osteocyte at a specific concentration. Since early stage is a necessary step in bone mineralization, the enhancing effect of *J.gendarussa* may stimulate the bone fracture healing.

#### **CONCLUSION**

As a conclusion, this study showed that *J.gendarussa* has potential in increasing the ALP activity in osteoblast cells. Therefore, J. gendarussa treatment may be favorable for the bone fracture healing, with a potential mechanism of stimulating the ALP activity in osteoblast cell.

### ACKNOWLEDGEMENT

We wish to thank Ministry of Higher Education (MOHE) for their funding under Research University Grant (VOT 4F344) and MyBrain 15 scholarship. We also thank Faculty of Biosciences and Medical Engineering, Universiti Teknologi Malaysia (UTM) for their facilities and services provided.