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Research Article

THE TOXICITY OF MAULI BANANA (*MUSA ACUMINATA*) STEM WATER EXTRACT ON BONE MARROW MESENCHYMAL STEM CELL *IN VITRO*

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ABSTRACT

Objective: Since mesenchymal stem cells (MSC) can differentiate into bone, cementum, and periodontal ligament, they can be used to treat aggressive periodontitis. The limited number of MSCs requires replenishment of growth factor in the cell culture process. Since growth factor is quite expensive, an alternative material is needed. Mauli banana stem has antioxidant and immunomodulatory properties. Methanol extract of Mauli banana stem is known to be toxic toward MSCs; therefore, another solvent with a non-toxic effect is needed, such as a water solvent. We analyzed the toxicity of Mauli banana stem water extract on MSC *in vitro*.

Methods: In this laboratory experimental (true experimental) study with a Post-test Only Control Group Design, MSC cultures were treated with Mauli banana stem water extract at 10, 20, 40, 60, 80, and 100 mg/mL dosages. One group without any treatment served as a control group and one was a media control group. Each group was incubated for 24 h and then was given 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide reagent and analyzed by an enzyme-linked immunosorbent assay (ELISA) reader.

Results: One-way analysis of variance showed a significant difference.

Conclusion: Mauli banana stem water extracts at 10, 20, 40, and 60 mg/mL were not toxic toward MSC *in vitro*, while dosages of 80 and 100 mg/mL dosage were toxic.

Keywords: Mauli banana stem extract, Mesenchymal stem cell, Toxicity.

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INTRODUCTION

Aggressive periodontitis is that which advances progressively in young patients, defined by the rapid loss of attachment [1]. This lesion is treated using bone graft. Bone graft is known to regenerate only half of the destroyed periodontal tissue, so the therapeutic approach has shifted to the use of stem cells for treatment [2,3]. Mesenchymal stem cells (MSCs) can differentiate into bone, cementum, and periodontal ligament, which enables their use for the treatment of aggressive periodontitis [4,5]. However, there is only one MSC per 10,000 nucleated cells, so a growth factor is necessary in addition to the cell culture process [6]. Growth factor is an exogenous material required for activation of the cell proliferation pathway so that the appropriate cell culture amount can be established. Exogenous phytochemical materials that found in herbal plant extracts can be used as an alternative growth factor to increase the number of MSCs. One such material is Mauli banana stem.

Mauli banana stem extract has the highest bioactive content of tannin (67.59%), followed by saponin (14.49%), alkaloid (3.44%), ascorbic acid (0.44%), flavonoid (0.25%), and lycopene (0.006%) [7]. Mauli banana stem extract is an antioxidant with the activity to bind heavy metal iron, hydrogen peroxide, and hydroxyl to decrease reactive oxygen species (ROS) [7,8]. ROS are secondary messengers in the intracellular signaling pathway, which regulates cell proliferation [9].

Mauli banana stem extract has the highest content of tannin having a polar property, so it can be dissolved in glycerol, alcohol, water, and acetone [7,10]. Carabelly *et al.*[11] proved that methanol extract of Mauli banana stem is toxic toward MSC. Hosein and Zinab [12] stated that tannin content extracted using methanol solvent is higher than that extracted using water solvent. We analyzed the toxicity of Mauli banana stem water extract on MSC *in vitro*. This study is expected to result in a standard dosage to be used for herbal medicine development of Mauli banana stem, which can be used as an alternative for growth factor to increase MSC numbers *in vitro*. This alternative agent is expected to be applied as alveolar bone destruction therapy in patients with aggressive periodontitis.

METHODS

Ethical clearance and permission to conduct this laboratory experimental study with a Post-test Only Control Group Design was obtained from the Ethics Committee of the Faculty of Dentistry, Lambung Mangkurat University (No.040/KEPKG-FKGULM/EC/IX/2017).

Mauli banana stem (SMK-PP Banjarbaru, South Kalimantan, Indonesia) was taken from a 10 cm root bump, was washed, was cut, and was placed in an oven at 60°C. Then, the dried Mauli banana stem was mashed using a blender and filtered with 35 mesh filter, was weighed to 50 g, and then was placed in a beaker glass to which 1000 mL Aquades was added and then heated to 50°C for 5 h. Then, it was stirred 3 times using a 500 revolution per minute stirrer at the beginning, middle, and near end of the heating process. The sample was cooled, was filtered, and was placed into an evaporator at 60°C for 2 × 24 h. After evaporation, the sample was placed in a 60°C water bath until viscous extract was obtained.

MSCs with 80% confluency were distributed in a 96-wheel microplate and divided into six treatment groups. Groups 1–4 consisted of MSCs given Mauli banana stem extract at 10, 20, 40, 60, 80, and 100 mg/mL dosages. Group 7 was not given any treatment (control group) and Group 8 was a media control. Each group was repeated 5 times. The microplate was incubated for 24 h, and then, the cells were washed with PBS and 25 µL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium reagent was added to each wheel. Then, the cells were incubated for

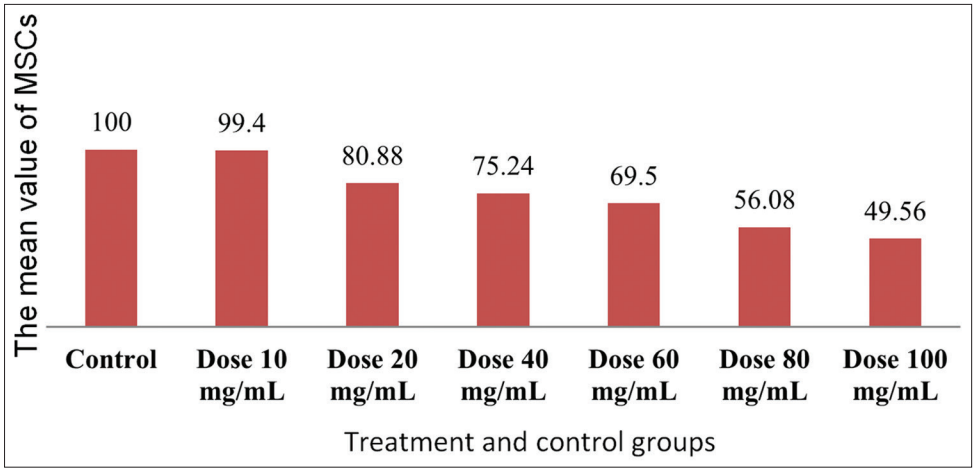

Fig. 1: Mean value of mesenchymal stem cells viability after Mauli banana water extract treatment

Table 1: Mean OD analyzed using the Games-Howell *post hoc* test between MSCs groups after treatment with Mauli banana stem water extract

Treatment Group	Control	Dose 10 mg/mL	Dose 20 mg/mL	Dose 40 mg/mL	Dose 60 mg/mL	Dose 80 mg/mL	Dose 100 mg/mL
Control		−0.003	−0.944*	−0.121*	−0.149*	−0.215*	−0.247*
Dose 10 mg/mL	0.003		−0.090*	−0.117*	−0.145*	−0.211*	−0.243*
Dose 20 mg/mL	0.944*	0.090*		−0.027*	−0.055*	−0.121*	−0.152*
Dose 40 mg/mL	0.121*	0.117*	0.027*		−0.028*	−0.094*	−0.125*
Dose 60 mg/mL	0.149*	0.145*	0.055*	0.028*		−0.066*	−0.097*
Dose 80 mg/mL	0.215*	0.211*	0.121*	0.094*	0.066*		−0.031*
Dose 100 mg/mL	0.247*	0.243*	0.152*	0.125*	0.097*	0.031	

*p>0.05, no significant difference

4 h at 37°C. After 4 h, the medium in the microplate was removed and replaced with dimethyl sulfoxide 200 µL/wheel. Then, the result was analyzed using an enzyme-linked immunosorbent assay (ELISA) reader with 595 nm wavelength.

RESULTS

The ELISA reader showed the optical density (OD) rate of the MSCs given Mauli banana stem water extract in each treatment and control group. Based on OD, the agent was said to be non-toxic if the cell viability percentage was >60%. Cell viability was calculated using the following formula [13]:

$$\text{Cell viability}(\%) = \frac{\text{OD treatment} - \text{OD media control}}{\text{OD cell ontrol} - \text{OD media control}} \times 100\%$$

Figure 1 shows that Mauli banana stem water extracts at 10, 20, 40, and 60 mg/mL were not toxic toward MSC *in vitro*, while dosages of 80 and 100 mg/mL were toxic. A one-way analysis of variance test revealed a significant difference. Hence, a Games-Howell *post hoc* test showed no significant difference between 10 mg/mL Mauli banana stem water extract and the control group Table 1. Therefore, the effective dosage of Mauli banana stem water extract that is not toxic toward MSC *in vitro* was 10 mg/mL.

DISCUSSION

Our results showed that Mauli banana stem water extracts at 10, 20, 40, and 60 mg/mL dosages were not toxic toward MSC *in vitro*, while dosages of 80 and 100 mg/mL were toxic. This result differs from that of Carabelly *et al*. [11], which showed that methanol extract of Mauli banana stem is toxic toward MSC. The difference in solvent choice influenced the number of extracts produced. Hosein and Zinab [12] stated that the tannin content in methanol extract is higher than that of water extract. Romadanu *et al*. [14] also stated that an extraction using methanol as the solvent has the highest yield. The high yield found in methanol solvent showed that this solvent can extract more bioactive components with higher polarity. Mauli banana stem extract has the highest content of tannin having a polar property [7,10]. Mauli banana stem extract using methanol solvent can extract more bioactive tannin component compared to water solvent. Based on the study of Ashok and Upadhayaya [15], tannin at high dose can cause toxic effects on the cells. High-dose tannin has pro-oxidant properties, so it can increase ROS [16,17]. The increased ROS activity can activate p53, so cell apoptosis can occur [18]. Tannin also is known for its property to increase Erk, which activates p53 for the apoptosis pathway through elevation of transcription among certain proapoptotic Bcl families, especially Bax [19,20]. Other than the difference in solvent choice, the difference in Mauli banana stem extract dosages also showed different toxicity results toward MSC *in vitro*.

Mauli banana stem extract has antioxidant properties, so it can decrease ROS [7,8]. ROS are secondary messengers in the intracellular signaling pathway, which regulates cell proliferation, differentiation, and apoptosis [9]. The effect of ROS on cell function depends on the dosage. At high dose, ROS can endanger the life of the cell and disturb physiologic function. However, ROS at low dosage also are important for cell signaling because it can modify redox-sensitive protein, which is involved in cell proliferation and differentiation [9]. The decreased ROS number caused by Mauli banana stem extract can activate the cell proliferation pathway directly or through the mitogen-activated protein kinase (MAPK) and Akt pathways [9,21].

Mauli banana stem extract has the highest bioactive content of tannin (67.59%) [7]. Mauli banana has the proanthocyanidin type of tannin (condensed tannin) [22], which can induce tyrosine phosphorylation in tyrosine kinase insulin receptors on the surface of MSCs to activate the MAPK/Erk and phosphoinositide 3-kinase (PI3K)/Akt/mTOR pathways [20,21,23,24]. Based on the study of Haston *et al*. [25], pERK1/2 staining can be detected in the marginal zone of Sox2+ stem cells, which shows that MAPK/Erk has a role in stem cell proliferation. Meanwhile, the PI3K pathway also has an important role for MSC

mitogenesis, proliferation, apoptosis prevention, and multipotent maintenance [26].

CONCLUSION

Mauli banana stem water extract at 10, 20, 40, and 60 mg/mL dosages is not toxic toward MSC *in vitro*, while dosages of 80 and 100 mg/mL dosage are toxic.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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