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The effect of bitter melon (*Momordica charantia* L.) ethanol extract on tibia bone fracture healing in the Wistar strain rats (*Rattus novergicus*) based on radiological assessment, histopathology, and alkaline phosphatase levels

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ABSTRACT

Introduction: Fracture is one of the most common health problem cases in the community. The goal of treatment for fractures is to restore the condition of the bone to pre-injury condition. This research aims to obtain a novel treatment for fracture healing. Bitter melon is a plant that is easily found and widely consumed in Indonesia. According to previous research, Bitter melon extract (*Momordica charantia* L.) can accelerate bone growth and repair after a fracture.

Method: In this study, parameters used to assess fracture healing were histological, radiological, and alkaline phosphatase levels. This study used Bitter melon extract with 70% ethanol. Research groups consist of 3 groups with different doses and 1 control group where each group consisted of 6 subjects. The dosage used is 200 mg/Kg BW (P1), 300 mg/Kg BW (P2), and 400 mg/Kg BW (P3). Before being treated the subjects underwent factorization procedure and then treated used using bandages and wooden

sticks for immobilization of fracture segments. Furthermore, the subjects were given Bitter melon extract twice a day for 84 days. Then checked according to the parameters seen. The data obtained are quantitative data which will be tested for normality and homogeneity first, then ANOVA test.

Result: Callus formation findings shows the P1 groups showed the highest mean of callus formation among treatment groups after induced by Bitter melon ethanol extract of 200 mg/kg BW. The results of LSD test in alkali phosphatase parameter between groups showed significant differences between the P1 group with controls ($p = 0.000$), P2 with controls ($p = 0.000$), P3 with controls ($p = 0.000$), P3 with P1 ($p = 0.000$) and P3 with P2 ($p = 0.021$)

Conclusion: There was a significant effect of the administration of bitter melon fruit extract on the formation of callus formation in animal model after osteotomy.

Keywords: Fracture, *Momordica charantia* L., histology, radiology, alkaline phosphatase.

Cite This Article: Rahman, S., Maryatun, Gan, A.K. 2020. The effect of bitter melon (*Momordica charantia* L.) ethanol extract on tibia bone fracture healing in the Wistar strain rats (*Rattus novergicus*) based on radiological assessment, histopathology, and alkaline phosphatase levels. *Bali Medical Journal* 9(1): 189-193. DOI:10.15562/bmj.v9i1.1743

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Received: 2020-01-19

Accepted: 2020-03-20

Published: 2020-04-01

INTRODUCTION

Fractures are common injury found in the community and have a long recovery time. The development of bone healing after fracture continues to evolved time to time. The main goal of early treatment of fractures is to improve the patient's quality of life by restoring the function of the extremities to pre-morbid condition. The principle of fracture treatment known as "4R" which are recognition, reduction, retaining and rehabilitation.¹⁻⁴

Macroscopically fracture healing can be observed by observing callus formation into mature bone. The callus formation begins to occur in the proliferation phase which occurs 3 days to 4 weeks after injury. The callus is formed from the outer layer of the periosteum where the layer is pushed by osteoprogenitor cells which developed into osteoblasts cells.⁵⁻⁷

Bitter melon also known as "bitter fruit" plant, grows mostly in the tropical region including in

Indonesia. Its easily cultivated and non-seasonal dependency to grow. Bitter melon contained many active triterpenoid compounds. According to the study of Hsu et al. triterpenoid content of bitter melon fruit has an estrogenic effect.⁸⁻¹⁰

Study by Gan et al. showed oral administration of Bitter melon ethanol extract with varies dosages of 100 mg/kg BW; 200 mg/ kg BW dan 300 mg/ kg BW induced callus formation significantly in animal model compared to control group ($p=0.025$).¹¹ This result supported by the study from Daramiana et al. which assessed the effect of oral administration of Bitter melon ethanol extract in osteoblast levels in tibia bone of animal mode after osteotomy. This study showed a significant increase in mean number of osteoblast in treatment groups compared to control group. ($p=0.000$).¹²

Based on the explanation above, the efficacy of triterpenoids compounds in Bitter melon showed

significant osteogenic activity which can be benefits in the initial process of bone healing of after in animal model through observation of callus formation and osteoblasts levels. Further research related to the administration of Bitter melon ethanol extract in experimental animals with longer study periods and applicable clinical parameters is the key to assessing the benefits of bitter melon extract administration as a part of treatment for fracture healing. This study aims to assess the benefits of Bitter melon ethanol extract administration with clinical parameters such as radiological, histopathological and biochemical assessment. Where, this clinical form of assessment more suitable to be applied later in humans.

MATERIALS AND METHODS

Study design

This study was an experimental laboratory study using a Completely Randomized Design (CRD) with the Posttest Only Control Design. This study was conducted in the Integrated Laboratory-Faculty of Veterinary Medicine and the Laboratory of Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala. This study was conducted in August-October 2018. The subject of this study was wistar strain male rats (*Rattus norvegicus*). The number of subject was determined using Frederer Formula. The subjects divided into 4 groups (3 treatment groups; 1 control group), each group consists of 10 subjects.

The subject of each group underwent osteotomy in diaphysis of tibia bone. The treatment groups given varies dosages of oral Bitter melon ethanol extract: 200 mg/kg BW (P1); 300 mg/kg BW (P2); and 400 mg/kg BW (P3). This study aims to assess the callus formation, osteoblast and alkaline phosphate levels.

Bitter melon Ethanol Extract Preparation

Bitter melon fruit was obtained from Teladan Village, Seulimum District, Aceh Besar Regency, 5 kg with an average weight of 200-300 grams/ per fruit. Bitter melon extract is made by washing it thoroughly and then drying it. The dried Bitter melon fruit is then mashed into powder and then extracted by maceration method. Bitter melon powder was macerated using 70% ethanol for 72 hours and then filtered using filter paper. The filtrate was evaporated with a vacuum rotary evaporator to remove ethanol solvents at 40°C until thick extracts were obtained.

Determination of Bitter melon ethanol extract dose was done by classifying doses based on the classification of low, medium and high with

modifications from previous study.^{11,12} Human conversion dose to Experimental Animal is 0.018. The dosage used for this study after calculations was 3.6, mg; 5.4 mg; and 7.2 mg.

Pre-Treatment Animal Care

The subjects were placed in a cage made of glass with size 15 × 40 × 15 cm for 5 subjects, equipped with feeding and drinking animals. The subject was adapted for 7 days before treatment in order to familiarize the test animal with experimental conditions and control its health. The animals are given 10g/200g BB pellet feed twice daily. During the adaptation, body weight and animal activity continue to be observed. The subject move actively and there is no weight less than 150 grams during and after the adaptation process.

Animal Treatment

The subject is undergone tibial osteotomy. The subject was anaesthetized using ketamine (80mg/kg BW) then disinfection in the operative area, then incisions extensively in the rat fascia. Then blunt dissection along the diaphysis of tibia. Osteotomy is done by using a saw. After osteotomy, the wound is closed, topical antibiotic was given. And installation of splints using wood sticks and bandages. Bitter melon ethanol extract was given twice a day orally using gastric tube for each group at 11.00 AM and 06.00 PM for 84 days starting on the first day after osteotomy is done in the study.

Radiological Assessment

The radiological assessment was performed in the 84th day after osteotomy. The equipment used was X-Ray machine with series CH00228908-98 Made in China with Fuji Medical X-Ray 100 NIF Film. Radiological assessment was done using Modified Lane and Shandu Score and Modified International Society of Limb Salvage Surgery (ISOLS) Score.

Bone Harvesting and Histological Specimen Preparation

After receiving treatment for 84 days, each subject will be sacrificed by anaesthesia inhalation and cervical fractures termination method. The histological specimen was obtained from previous site of osteotomy. The specimen was stained using Hematoxyllin-Eosin staining.

Alkaline Phosphatase Levels Measurement

The principle for histochemical detection of alkaline phosphatase is measure the catalyze process of 4-nitrophenyl phosphate and 2-amino-2-methyl-1-propanol (AMP) to 4-nitrophenol. The increase

in 4-nitrophenol is measured by photometry at a wavelength of 405 nm.

Data analysis

The data obtained was undergone the normality test (Shapiro-Wilk) and the homogeneity test (Levene). If these two tests are fulfilled, then ANOVA tests are carried out to see whether or not there are significant differences between treatment groups. If the normality test and data homogeneity are not fulfilled, then data underwent data transformation. If the data is still not normally distributed and not homogeneous, then the Kruskal-Wallis test is used. If the ANOVA test showed significant differences, then significance difference between treatments was measured using the Least Significant Difference (LSD) test.

RESULTS

The results of subject weight measurements before experiment in Faculty of Veterinary Medicine, University of Syiah Kuala can be seen in [Table 1](#).

Measurement of callus formation in the tibia bone after osteotomy was done in 84th days. The data obtained was callus width of bone tibial osteotomy measured by radiological assessment. The results of the callus formation in each group showed in [table 2](#).

[Table 2](#), showed that each treatment group had different mean of callus size. The P1 groups showed the highest mean of callus formation among treatment groups after induced by Bitter melon ethanol extract of 200 mg/kg BW. Then followed by the P2 group, P3 group, while the highest results were

Table 1 Pre-treatment subject weight

Subject	Group			
	control	P1	P2	P3
1	162 g	164 g	158 g	154 g
2	160 g	171 g	161 g	157 g
3	158 g	169 g	167 g	161 g
4	153 g	152 g	155 g	158 g
5	164 g	156 g	156 g	152 g
6	168 g	161 g	162 g	171 g
7	164 g	194 g	150 g	175 g
8	157 g	170 g	176 g	165 g
9	170 g	164 g	175 g	162 g
10	164 g	165 g	171 g	180 g

Table 2 The effect of Bitter melon Ethanol Extract in Callus Formation in each group after 84 experiment days

Subject	Group			
	Control	P1	P2	P3
1	0.9	0.5	0.47	0.53
2	0.7	0.5	0.67	0.48
3	0.9	0.6	0.6	0.4
4	0.8	0.7	0.45	0.44
5	0.9	0.8	0.5	0.4
6	0.8	0.5	0.45	0.48
7	0.9	0.5	0.5	0.42
8	1	0.67	0.6	0.4
9	0.8	0.6	0.55	0.45
10	0.75	0.65	0.5	0.42
Total	8.45	6.02	5.29	4.42
Mean	0.845	0.602	0.529	0.442
SD	0.896	0.104	0.074	0.434

Table 3 The effect of Bitter melon Ethanol Extract in Alkaline Phosphatase Levels in each group after 84 experiment days

Subject	Group			
	Control	P1	P2	P3
1	375	306	309	728
2	187	532	352	490
3	347	396	461	498
4	269	356	518	455
5	358	546	334	537
6	206	382	595	451
7	284	334	339	476
8	259	452	487	593
9	342	427	435	398
10	225	388	378	493

Table 4 The result of Least Significant Difference (LSD) Test in Alkaline Phosphatase Levels between each groups

	Control	P1	P2	P3
Control	-	-	-	-
P1	0.000*	-	-	-
P2	0.000*	0.51	-	-
P3	0.000*	0.000*	0.021*	-

found in the control group. The effect of bitter melon ethanol extract

Data were normally distributed and homogeneous. Therefore, data analysis is continued by using the ANOVA test. The one-way ANOVA test showed differences between each group ($p = 0.000$). The difference between each treatment was analysed using Least Significant Difference (LSD) test in Table 4.

The results of LSD test showed significant differences between the P1 group with controls ($P = 0.000$), P2 with controls ($P = 0.000$), P3 with controls ($p = 0.000$), P3 with P1 ($p = 0.000$) and P3 with P2 ($P = 0.021$), except in the P2 with P1 ($p = 0.51$). In the results of the analysis also showed higher the dose of Bitter melon Ethanol Extract increased the bone growth which characterised by the less callus formation that appears from the results of radiological examination (Table 4).

DISCUSSION

The administration of Bitter melon extract in animal models showed significant effect at each concentration compared to control group. The previous studies stated that Bitter melon fruit has significant amount of phytoestrogen. Based on the

theory, it is said that estrogen influences the formation of osteoblasts and osteoclasts which become an influential component of bone formation and remodelling.

This study showed oral administration of Bitter melon Ethanol extract results in significant difference of callus formation between treatments and control groups. In the treatment groups, subjects that were given Bitter melon extract at a dose of 100 mg/kg BW showed no significant effect in callus formation, whereas in the group of Bitter melon extract at a dose of 200 mg/kg BW and 300 mg/kg BW, the statistical analysis showed significant relation between the administration of the dosing and callus formation.

This results are consistent with the previous study by Cevik et al.,¹³ which stated that Bitter melon fruit has an active effect on the Estrogen Receptor- β . In addition, study by Hsu et al.,¹⁰ mentioned that Bitter melon fruit has triterpenoid content which is act as phytoestrogen that has a similar phenolic structure to the estrogen, and has properties that can be bound to genes on chromosomes that can control Estrogen Receptor- β (ER- β).

In the process of fracture healing, estrogen plays important role in bone growth and remodelling.^{14,15} A study by Beil et al.¹⁵ showed there is significant difference in callus formation on the 14th day between animal models that induced by estrogen and other groups that performed ovariectomy so that there is no. The results of the Kawiyana study stated that estrogen plays a role in increasing osteocyte formation and stimulating osteoprogenitor (OPG) and Transforming Growth Factor β (TGF- β) in osteoblast cells to form bone matrix.¹⁶ Osteoprogenitor cells will differentiate and proliferate into osteoblast cells that secrete the osteoid matrix resulting in the process of bone formation, namely primary and secondary ossification.¹⁷

Alkaline phosphatase is the most commonly used bone growth indicator. Alkaline is closely related to osteoblast in bone and indicates the osteoporosis if the number is significantly decreased.¹⁸ The role of the alkaline phosphatase enzyme in the mineralization process is that prepare an alkaline condition in the osteoid tissue so that calcium can be easily deposited on the matrix. In addition, this enzyme caused an increase in phosphate concentration, so that calcium-phosphate bonds are formed in the form of hydroxyapatite crystals which the crystals will eventually settle in the bone matrix.¹⁹

Bone remodelling begins with a process of blood clot formation (hematoma). In this phase, granulation tissue formed contains Osteoprogenitor (OPG) cells which will differentiate into chondroblasts and osteoblasts. The phase is called the proliferation

phase until the hematoma absorbed so that the new capillary develops in the fracture area. The next phase is callus formation, which is characterized by thickening of the bone caused by osteocyte cell formation by osteoblasts. The layer will continue to thicken until fuse from the two separate bone fragments.^{20,21}

CONCLUSIONS

There was a significant effect of the administration of bitter melon fruit extract on the formation of callus formation in animal model after osteotomy. Administration of Bitter melon extract dose of 400 mg/Kg BW had a greatest influence on bone growth in this study. This is indicated by the less callus formation on radiological examination compared to other groups.

CONFLICT OF INTEREST

The author declares there is no conflict of interest regarding publication of current article.

FUNDING

Current study doesn't receive any specific grant from government or any private sectors.

ETHICAL CLEARANCE?

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