Effectivity of Low Intensity Pulsed Ultrasound on Rabbit Femur Post Orif Plate and Screw

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A femoral fracture is a trauma with a high mortality rate. Long bone fracture cases frequently related to missing bone segments, that can disrupt the bone healing. The process of defect fracture healing requires internal and external mechanical stimulation, external stimulation can be stimulated using low-intensity pulsed ultrasounds. Clinical studies have shown a significant positive effect from LIPUS in the treatment of non-union fractures and accelerated fracture repair compared to fractures treated with a placebo of 38%. The purpose of this study was to determine the effectiveness of LIPUS on the healing of defect femoral fractures in rabbit post-ORIF plate and screw-based on radiological measurements. This study is a comparative, unpaired laboratory experimental study with two treatment groups and one control group. The treatment group consists of group I that was given LIPUS 30 mW/cm2 for 10 minutes per day and Group II 30 mW/cm2 for 20 minutes per day, Group III without LIPUS treatment as the control group. Radiological assessment was performed to assess callus formation based on the Tiedemann score. After that data is collected, data editing, coding, entry were carried out and analyzed statistically. There were significant differences between treatment group and control group towards callus diameter formation, between-group I, II and control with mean of Tiedemann in each group 97.76; 169.83; 76.88; with p-value < 0.05. LIPUS can enhance fracture healing in rabbit femur defects based on radiography measurement.

Keywords: Fracture; Femur; Healing; Mechanical; LIPUS; Radiological.

A femoral fracture is a trauma with a high mortality rate. The incidence of femoral fractures in America is approximately 13 cases per 100,000 people per year.[1,2] In Indonesia, it is reported that the incidence of fractures every year is 11.10 cases per 1,000 people per year, with an incidence of 11.67 cases per 1,000 people per year in men and 10.65 cases per 1,000 people per year in women. In children and adults, femoral fracture events are associated with low energy mechanisms, whereas in the young adult the mechanism is high energy.[1-3] Long bone fractures are often accompanied by bone defects, this can interact with the healing process. If internal fixation is done to this fracture, then the defect will interrupt the healing process. The incidence of long bone fractures with defects is 13.9%.[4,5]

Fracture healing is a complex process. Clinically, it is necessary to reduce and stabilize shifting fractures.4 The biological process of fracture healing consists of several different phases. This phase includes the inflammatory phase with cell proliferation, the chondrogenic phase with cartilage hypertrophy and angiogenesis, and the osteogenic phase with cartilage replacement with woven bone and remodeling.4 Fracture healing is influenced by external (biomechanical) and internal (biological) stimuli. Biological interventions such as the use of allogenic bone graft, substitute substances for a bone graft, medical, while external stimuli in the form of mechanical and physical interventions, such as static and dynamic methods for stabilization in operative actions, and the use of noninvasive actions such as electromagnetic and ultrasound.6-8

Ultrasound is a source of mechanical energy delivered as acoustic pressure waves outside the range of human hearing.5 Ultrasound has a variety of medical applications, ranging from diagnostic tools to therapeutic agents.5 Usually, at low intensities (0.5-50 mW/cm2), ultrasound acts as a diagnostic modality, whereas at higher intensities (0.2-100 mW/cm2), ultrasound has a therapeutic effect by producing heat energy.5 Although preliminary studies have found high-intensity ultrasound (0.2-100 mW/cm2) inhibits bone healing, several recent studies studying low-intensity pulsed ultrasound (LIPUS) in the range of diagnostic intensity have shown more beneficial effects.[5,6]

Clinical studies have shown a significant positive effect of LIPUS in the treatment of fracture cases that fail to connect.4 Other studies investigating the application of LIPUS in conservative tibia fractures and distal radius fractures both show a 38% acceleration in fracture repair. Gebauer reported an accelerated rate of fracture healing by 85% after 5 months, while Nolte et al reported the effect of LIPUS on accelerating fracture healing by 86% in an average time of 5.5 months.[6-10]

Long bone fractures, especially the femur, are a fracture that needs peculiar treatment, so that the healing process of the bone is not disrupted which causes the bones to not attach. The bone healing process requires sufficient internal and external stimuli. External stimulation (biomechanics) can accelerate the fracture healing process, one of which is using low-intensity ultrasound. Low-Intensity Pulsed Ultrasound has been known to accelerate the fracture healing process. What is the role of Low-Intensity Pulsed Ultrasound as an alternative measure to the formation of hard callus in rabbit femur defects after ORIF plate and screw-based on radiographic measurements? In this study, we will examine the effects of ultrasound stimulation on hard callus formation in rabbit femur defects post ORIF plate and screw-based on radiographic measurements.

MATERIALS AND METHODS

This study is an experimental laboratory test in animals with a simple randomized sampling method. The object of this study used subjects in the form of experimental animals, namely New Zealand white rabbits that met the inclusion criteria. Research samples in the case and control groups were taken by completely randomized design methods that met the inclusion and exclusion criteria. The sample size of experimental animals in this study was a minimum of 9 animals for each group based on the Federer formula. The addition may be done with a condition of 10% of the minimum number of experimental animals used (1 animal). From the calculation of sample size/research subjects, the minimum sample/research subject is ten, so to anticipate the dropout, the number of research subjects is set at 10 adult male rabbits per group with the total sample in this study is 15 white male New Zealand rabbits.

The case group of this study was divided into three groups, so the total number of research subjects were 15 male rabbits from which the hind legs were taken both left and right (femur bones). Each case group was coded as follows; (Group I) The group is treated with LIPUS therapy with 30 mW/cm2 given for 10 minutes per day, (Group II) The group that was treated with LIPUS therapy with 30 mW/cm2 given for 20 minutes per day, (Group III) the control group.

The inclusion criteria in this study were: 1) New Zealand white rabbits; 2) male sex; 3) young adult rabbits (8 months on average); and 4) rabbit weight ranges from 3000-3500 grams. Exclusion criteria in this study were sick rabbits and appeared to be inactive during the adaptation period before treatment was carried out during the study.

The study was carried out in the Medical Research Unit Laboratory of Medical Faculty of UNPAD Eijkman Street No. 38 Bandung; and at the Faculty of Pharmacology and Therapies at the Medical Faculty of UNPAD Eijkman Street No. 38 Bandung. The research period is from March 2019 – April 2019.

A random sample of 30 rabbit feet was taken. Samples were divided into 3 groups. Each group consists of 10 rabbit feet. In all groups the right/left femur was made fractured, with a 5 mm defect made, and fixed with a miniplate implant, the wound was closed again and given injection therapeutic antibiotics. The first group was given LIPUS therapy 30 mW / cm2 for 10 minutes every day. The second group was given LIPUS therapy 30 mW / cm2 for 20 minutes every day. The third group is the control group. Anteroposterior and lateral radiological examinations of rabbits were performed at weeks 3 and 5.

After the data is collected, editing, coding, and entry are carried out. Data processing uses the Excel 2010 for Windows and the SPSS for Windows. A descriptive analysis was performed. Bivariate analysis is carried out using: a normality test and data distribution are carried out to determine whether the parametric or nonparametric analysis will be used. Numerical variable comparative hypothesis testing was carried out for more than two groups using One Way ANOVA for parametric tests or Kruskal-Wallis for nonparametric tests.

This research was conducted using experimental methods and interventions on experimental animals, so we need an ethical clearance by applying the 3R principle (Replacement, Reduction, Refinement). Ethical clearance must be obtained from the Medical Research Ethics Commission Unpad – RSHS. New Zealand white rabbit experimental animals are kept in the Laboratory of Pharmacology Clinic of Medical Faculty of Unpad and were given ad libitum for food and drink. The Experimental rabbits are kept in cages of 80cm x 60cm x 60cm filled with 1 rabbit in each cage. To reduce pain during surgery, intramuscular ketamine is given first so that the rabbit is free from pain. Before bone tissue sampling is done, the rabbit was terminated first.

RESULTS

The procedure of the study was to take a sample of 30 rabbit feet randomly. Samples were divided into 3 groups. Each group consists of 10 rabbit feet. In all groups the right/left femur was

Table 1. Research Data

Variable	Group	N	Minimum	Maximum	Mean	SD
Callus Diameter	LIPUS 10 min/day	10	2.20	2.80	2.51	0.202
week 3	LIPUS 20 min/day	10	2.20	3.40	2.87	0.353
	Control	10	1.40	1.90	1.64	0.190
Callus Diameter	LIPUS 10 min/day	10	3.10	3.80	3.46	0.232
week 5	LIPUS 20 min/day	10	3.60	4.50	4.01	0.260
	Control	10	1.90	2.80	2.40	0.263
Callus Volume	LIPUS 10 min/day	10	44.70	62.20	50.60	5.931
week 3	LIPUS 20 min/day	10	69.30	104.50	88.81	11.640
	Control	10	27.80	46.70	36.09	6.475
Callus Volume	LIPUS 10 mn/day	10	85.70	120.60	97.76	12.335
week 5	LIPUS 20 mn/day	10	118.90	200.40	169.83	24.064
	Control	10	62.70	92.50	76.88	9.602
Tiedemann	LIPUS 10 min/day	10	2	4	3.20	0.632
week 3	LIPUS 20 min/day	10	2	5	3.80	0.919
	Control	10	1	4	2.30	0.949
Tiedemann	LIPUS 10 min/day	10	3	5	3.80	0.632
week 5	LIPUS 20 min/day	10	3	6	4.30	0.949
	Control	10	2	4	2.80	0.632

fractured, with a 5 mm defect was made, and fixed with a miniplate implant, the wound was closed again and given injection therapeutic antibiotics. The first group was given LIPUS therapy 30 mW / cm2 for 10 minutes every day. The second group was given LIPUS therapy 30 mW / cm2 for 20 minutes every day. The third group was a control group, then an anteroposterior and lateral radiological examination of the rabbit femur was done at weeks 3, and 5. A disguised radiology assessment was carried out for callus formation based on Tiedeman scores. The data obtained are shown in Table 1. below.

In callus diameter of group I on the third week (LIPUS 10 min/day) minimum value of 2.20, a maximum of 2.80, a mean value of 2.51 with a standard deviation of 0.202 was obtained. In the second group (LIPUS 20 min/day) minimum value of 2.20, a maximum of 3.40, an average of 2.87 and a standard deviation of 0.353 was obtained. In the control group, a maximum value of 1.40, a maximum of 1.90, a mean value of 1.64 with a standard deviation of 0.190 was obtained. The callus diameter of group I on the fifth week (LIPUS 10 min/day), a minimum value of 3.10, a maximum of 3.40, a mean value of 3.46 with a standard deviation of 0.073 was obtained. In Group II (LIPUS 20 min/day), a minimum value of 3.60, a maximum value of 4.50, a mean of 4.01 and a standard deviation of 0.26 was obtained. For the control group, the minimum callus diameter was 1.90, the maximum diameter was 2.80 with a mean value of 2.40 and a standard deviation of 0.083.

The acquisition of callus volume data in the third week of group I was at a minimum of 44.70, a maximum of 62.20, the mean value of 50.60 with a standard deviation of 5.931. Group II, the minimum callus volume value was 69.90, the maximum was 104.50 and the mean value was 88.81 with a standard deviation of 11.64. While the control group had a minimum value of 27.80, a maximum of 46.70, a mean of 3.09 with a standard deviation of 9.61. For week five of the treatment on the group I, callus volume increased with a minimum value of 85.70, a maximum of 120.60, a mean of 97.76 and a standard deviation of 12.33. The callus volume on group II had a minimum value of 18.04, a maximum of 200.40, a mean of 189.63 and a standard deviation of 24.06. The control group had a minimum callus volume value of 62.70, a maximum of 92.50, a mean of 76.88 with a standard deviation of 9.60.

The acquisition of callus volume data in the third week of group I was at a minimum of

Table 2. Mean Difference Test for Callus Diameter on Week 3 and Week 5

Variabel	Perlakuan	N	Mean	P-value
Diameter.Kalus	LIPUS 10 mnt/hr	10	2.510±0.203	0.000*
Week 3	LIPUS 20 mnt/hr	10	2.870±0.353	
	Kontrol	10	1.640±0.189	
Diameter.Kalus	LIPUS 10 mnt/hr	10	3.460±0.232	0.000*
Week 5	LIPUS 20 mnt/hr	10	4.010±0.260	
	Kontrol	10	2.400±0.263	

*Anova test

Table 3. Difference Test in Mean Callus Volume on Week 3 and Week 5

Variable	Treatment	N	Mean	P-value
Callus Volume	LIPUS 10 min/day	10	50.600±5.93	0.000*
Week 3	LIPUS 20 min/day	10	88.810±11.64	
	Control	10	36.090±6.47	
Callus Volume	LIPUS 10 min/day	10	97,760±12.34	0.000*
Week 5	LIPUS 20 min/day	10	169,83±24.06	
	Control	10	76.88±9.60	

*Kruskal Wallis Test

44.70, a maximum of 62.20, the mean value of 50.60 with a standard deviation of 5.931. Group II, the minimum callus volume value was 69.90, the maximum was 104.50 and the mean value was 88.81 with a standard deviation of 11.64. While the control group had a minimum value of 27.80, a maximum of 46.70, a mean of 3.09 with a standard deviation of 9.61. For week five of the treatment on the group I, callus volume increased with a minimum value of 85.70, a maximum of 120.60, a mean of 97.76 and a standard deviation of 12.33. The callus volume on group II had a minimum value of 18.04, a maximum of 200.40, a mean of 189.63 and a standard deviation of 24.06. The control group had a minimum callus volume value of 62.70, a maximum of 92.50, a mean of 76.88 with a standard deviation of 9.60.

Data Analysis

The data analysis technique used in this study is the One Way Anova because the data in this study are normally distributed data, while the data that are not normally distributed used the Kruskal Wallis test. Continuation of the One Way Anova test is if there are significant differences, then proceed with the Post Hoc test, while the Kruskal Wallis test with the Man Whitney Test with a significance of 5%.

ANOVA Test for Callus Diameter on Week 3 and Week 5

ANOVA test was used to determine differences in callus diameter of group I, group II and control group together. ANOVA test results for callus diameter of week 3 and week 5 can be seen in the following table.

Based on the presentation of Table 2. the mean value of callus diameter at week 3 of the group I (LIPUS 10 min/day) was 2.510 ± 0.203, group II (LIPUS 20 min/day) was 2.870 ± 0.353 and the control group was 1.640 ± 0.189 with a value of $p = 0.000 < 0.05$ which means there is a significant difference in the mean value of the 3rd week callus diameter between the three treatment groups.

The fifth-week callus diameter of group I (LIPUS 10 min/day) was 3,460 ± 0.232, group II (LIPUS 20 min/day) was 4,010 ± 0.260 and the control group was 2,400 ± 0.263 with a p-value = 0,000 <0.05 which means there was a significant difference in the mean value of the 5th-week callus diameter between the three treatment groups. Because the callus diameter test results are significant, the Post Hoc Test is continued to determine which treatment is more effective.

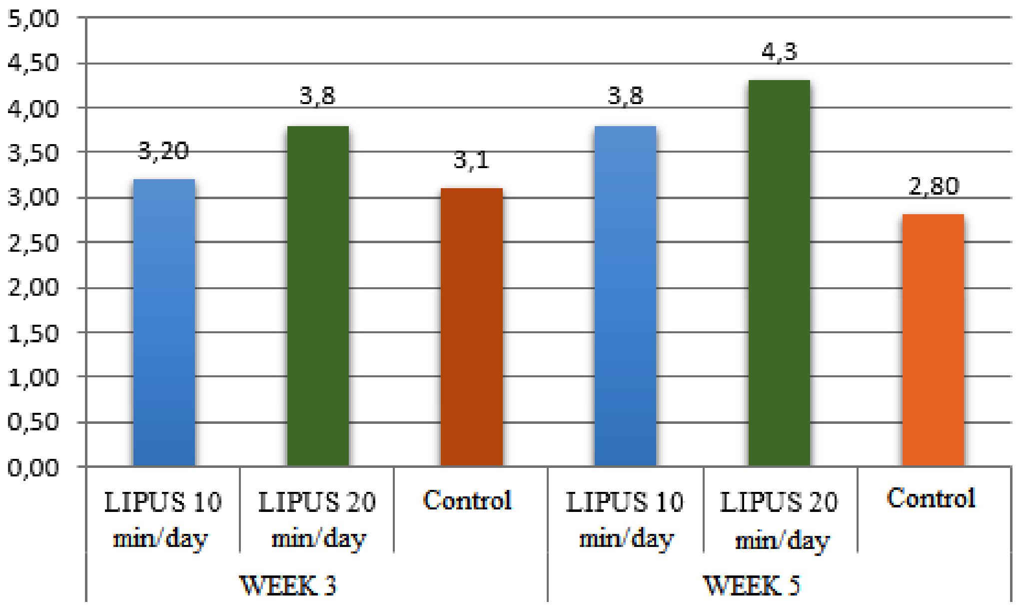

Fig. 1. Mean Value of Tiedmann Week 3 and 5. It is showed that the group that was treated with LIPUS therapy with 30 mW/cm² given for 20 minutes per day is more effective between the three treatment groups

Kruskal Wallis Test of Callus Volume on Week 3 and Week 5

Based on Table 3. the mean callus volume at week 3 of group I (LIPUS 10 min/day) was 50,600 ± 5.93, group II (LIPUS 20 min/day) was 88,810 ± 11.64 and control group was 36,090 ± 6.47 with p-value = 0.000 <0.05 which means there is a significant difference in the mean value of the third-week callus volume between the three treatment groups.

The fifth-week callus volume of the treatment group I (LIPUS 10 min/day) was 97.760 ± 12.34, the treatment group II (LIPUS 20 min/day) was 169.83 ± 24.06 and the control group was 36.090 ± 6.47 with a value of p = 0.000 <0.05 which means that there is a significant difference in the mean callus volume on week 5 between the three treatment groups. Because the callus volume test results are significant, the Mann Whitney test is continued to find out which treatment is more effective.

Kruskal Wallis Test of Tiedmann data on Week 3 and week 5

Based on Figure 1. Tiedmann's mean value obtained in week 3 of treatment group I (LIPUS 10 min/day) was 3.20 ± 0.63, treatment group II (LIPUS 20 min/day) was 3.80 ± 0.92 and control group was 3.10 ± 1.03 with a value of p = 0.005 <0.05 which means there is a significant difference in the mean value of Tiedman on week 3 between the three treatment groups.

Tiedman value on week five of the treatment group I (LIPUS 10 min/day) was 3.80 ± 0.63, treatment group II (LIPUS 20 min/day) was 4.30 ± 0.95 and the control group was 2.80 ± 0.63 with a p-value = 0.000 < 0.05 which means there is a significant difference in the mean value of Tiedman week 5 between the three treatment groups. Because the Tiedman test results are significant, the Mann Whitney test is continued to find out which treatment is more effective.

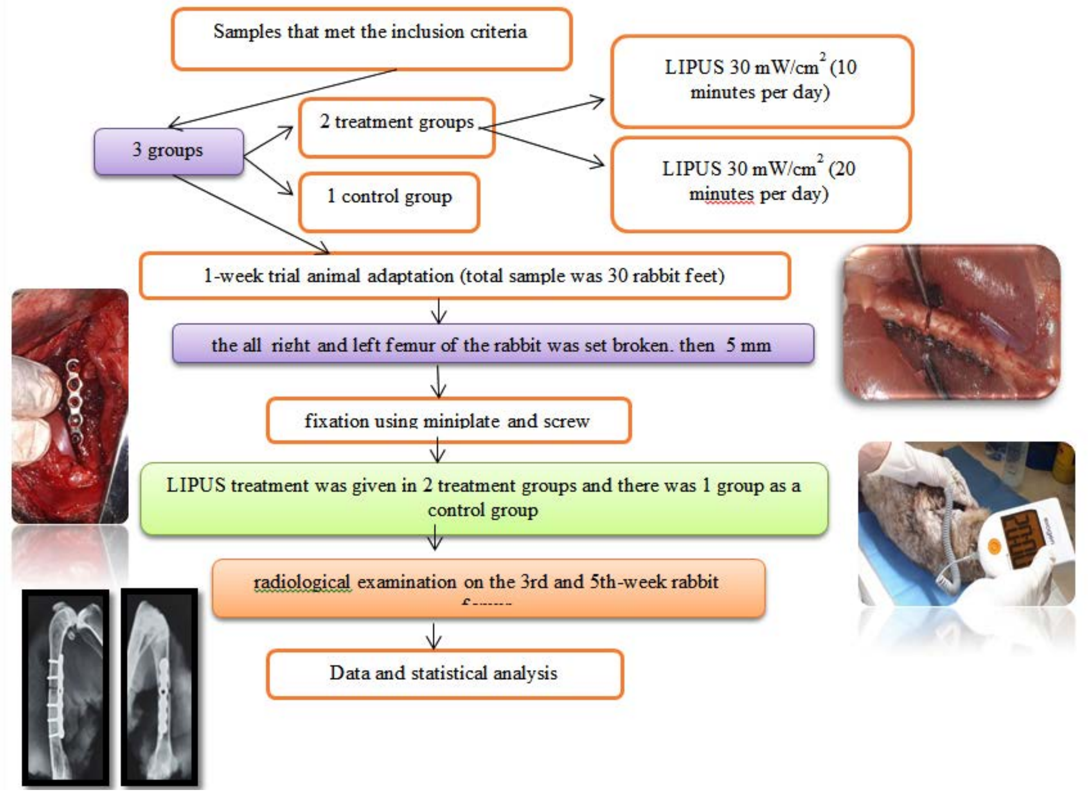

Fig. 2. Research procedure

DISCUSSION

The results showed that there were significant differences between the three treatments on callus diameter, callus volume, and Tiedmann score. From the results of the comparison between treatments obtained a significant result between the treatment group I with the control group and treatment group II with the control but the more effective is treatment II because the mean value on the callus diameter, callus volume, and tiedmann produced higher than group I, so it can be concluded that the stimulation of Low-Intensity Pulsed Ultrasound increases callus formation in the healing process of rabbit femur fractures based on radiographic measurements.[10-13]

LIPUS treatment for 20 minutes shows an acceleration of 38% success rate of nonunion 86%. Ultrasound is a source of mechanical energy delivered as acoustic pressure waves outside the range of human hearing. Ultrasound has a variety of medical applications, ranging from diagnostic tools to therapeutic agents. Usually, at low intensities (0.5-50 mW / cm2), ultrasound acts as a diagnostic modality, whereas at higher intensities (0.2-100 mW / cm2), ultrasound has a therapeutic effect by generating heat energy. LIPUS, in particular, functions as a potential noninvasive therapy for fracture healing. Waves given by LIPUS induce micromechanical stress at the fracture site, culminating in the stimulation of various molecular and cellular responses involved in fracture healing.[11,12-15]

Previous clinical studies have reported a lot about the use of low-intensity ultrasonic waves to accelerate fracture healing with varying results (acceleration of 28% – 45%). Using Wolff's basic law and the effect obtained is determined by the amount of energy received by the target the more energy received to a certain tolerance level the greater the effect of accelerating callus formation.[11,16,17]

Similar results were also reported by Hantoko et al, namely in the tibia fracture group treated conservatively and with ultrasound compared to the control group, the degree of callus thickness was assessed from the results of X-ray examination, the treatment tended to be higher than in the control. Besides, it can also be seen that the difference in the degree of callus thickness at the end of the week is higher than that of the beginning week or in other words, the difference is getting more and more real. Giving low-intensity ultrasonic waves with a duration of 15 minutes, as much as 10 times and with 3 days interval in tibia fractures that were managed conservatively (repositioned and plastered) proved to be able to accelerate callus formation ($p = 0.069$).[11,14]

Malizos et al. Concluded that LIPUS therapy can accelerate the healing process of fractures in nonunion patients, LIPUS therapy can induce fracture healing, and reduce the need for operative measures.[14,15] Although the average time needed to recover is around 5 months. Red staining with alizarin has verified the increased formation of calcium nodules in osteoblasts after LIPUS intervention. This causes an increase in stiffness and thickness of bone callus. Endochondral ossification enhanced by LIPUS also results in wider callus formation with increased mineral deposition.[15,17]

LIPUS also plays a role in stimulating the differentiation of several cells involved in fracture healing. These cells include chondroblasts, mesenchymal cells, fibroblasts, and osteoblasts. LIPUS increases the expression of aggrecan, a cartilage structural macromolecule that acts as a potent stimulant from chondrogenesis. Increased concentrations of aggrecan cause the acceleration of chondroblast to chondrocytes. More Chondrocytes in the area of injury cause increased release of chondroitin sulfate, an important component that supports the structure of cartilage and bone. Besides, due to the mechanism of cavitation, LIPUS increases blood pressure at the fracture site due to increased vascular permeability.[8,9,13,17]

The limitations of this study are the difficulty to readjust and maintain the 5 mm gap, and the lack of parameters used to evaluate fracture healing, and the anatomic and biomechanical pathology examination should be added.

CONCLUSION

LIPUS treatment group (LIPUS 10 min/day and LIPUS 20 min/day) can enhance the healing process of rabbit fractures post ORIF Plate and Screw based on radiological measurements compared to the control group. Further research is needed on clinical application, optimal dosage and assessment of the role of LIPUS as adjuvant therapy in accelerating fracture healing, especially at Dr. Hasan Sadikin Hospital Bandung.

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