Advances in Health Sciences Research, volume 37

Proceedings of the 12th Annual Scientific Meeting, Medical Faculty, Universitas Jenderal Achmad Yani, International Symposium on "Emergency Preparedness and Disaster Response during COVID 19 Pandemic" (ASMC 2021)

The Influence of Cissus quadrangula Salisb Extract to Bone Length and the Number of Osteoblast in Femur of DDY Mice

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***Abstract*—*Cissus quadrangula Salibs* have been proven to have an influence on the growth of bone and the healing process in fractures and prevent osteoporosis. *Cissus quadrangula Salibs* has been widely used as traditional medicine and has proven to increase osteoblast activity, and could accelerate the recovery of bone fractures. The study aimed to assess the effect of the stem of *Cissus quadrangula Salibs* on the growth of bone length and the number of osteoblast cells in mice femur. This study used a comparative method with a completely randomized design of 24 mice which were divided into 4 groups. P0 is a control group that is only given standard food, while P1 to P3 are the treatment groups given *Cissus quadrangula Salibs* extracts at doses of 500 mg/Kg BW, 700 mg/Kg BW, and 900 mg/Kg BW. The extract was given for 30 days, then measured the length of the bone and the number of osteoblasts on the metaphyseal plate of mice femur. Data were analyzed using One Way ANOVA and Post Hoc Duncan's test with a 95% degree of confidence. The results showed that *Cissus quadrangula Salibs* extract at a dose of 700 mg/Kg BW and 900 mg/Kg BW affects the length of the femur and the number of osteoblasts was significantly higher (p <0.005) than the control group. It can be concluded that *Cissus quadrangula Salibs* extract has the effect of increasing the length growth and number of osteoblasts Os. Femur in DDY strain mice.**

Keywords*—*Cissus quadrangular Salibs, bone length, osteoblast, femur

I. INTRODUCTION

Bone is a mineral structure formed from cells, vessels, and calcium crystalline compounds (hydroxyapatite). Bone consists of a wide variety of cells and an extracellular matrix that mineralizes from calcium crystals, which make bones strong. Bones will dynamically and continuously perform the remodeling process, namely the process of composing new bone tissue and remodeling old bone tissue. Throughout life, bones undergo periodic remodeling which includes the resorption process and the formation of this process is regulated by 3 main cells in the bone, namely osteoblasts, osteoclasts, and osteocytes. Osteoblasts function as bone formers, osteoclasts as bone resorption, and osteocytes, which are mature forms of osteoblasts and are the main bone-forming cells, buried in the bone matrix. Osteoclasts and osteoblasts are the main cells during the bone remodeling process and are closely linked in this process [1- 4].

Bone growth and formation are influenced by several factors, such as hormones, nutritional intake, age, physical activity, minerals, especially calcium, phosphorus, magnesium, vitamin D, the hormone estrogen, and the physical activity given to the bones. The hormone estrogen is the most important agent to maintain the balance of remodeling. Estrogen, which is known as an anti-resorptive agent, can inhibit bone resorption by osteoclasts. Besides being found in the body, estrogen can also be found in plants in the form of phytoestrogens. Phytoestrogens are endogenous estrogen-like substrates that have an effect on bones as estrogen, as an anti-resorptive by suppressing osteoclast activity, and also controls osteoclastic activity by suppressing the production of interleukin-6 (IL-6) produced by osteoblasts, thereby increasing bone length growth [3-8]. Cissus quadrangula SalibsLinn is one of the plants that have high levels of phytoestrogens [9,10].

Cissus quadrangula Salibs Linn is a plant belonging to the *Vitaceae* family which can be found in the Aceh region of Indonesia. This plant contains phytoestrogens, calcium, and

phosphate which are useful in the process of bone osteogenesis. The calcium content in this plant is useful in helping calcium fulfillment in the process of osteogenesis while phytoestrogens and phosphates play a role in the process of increasing calcium levels. Calcium is the important micronutrients which functions in the formation of bones and teeth, regulates blood clotting, muscle contraction in the body containing 1-2g calcium, more than 90 percent calcium contained in the bone. The amount of calcium needed by the human body varies. In Asia, most adults consume less than 500mg/day of calcium [10]. Adequate Calcium intake has effects on bone health such as higher bone mineral accretion at early ages and prevention of osteoporosis. The study in 2011 showed that giving *Cissus quadrangula Salibs* stem extract at a dose of 750 mg/kg BW per day orally can improve the bone density of female rats during the growth period so that it can be used as an anti-osteoporosis seen from the increase in bone density osteoblasts with the most effective time of 120 days [10-13].

Cissus quadrangula Salibs stem extract increase bone cell proliferation assessed from an increase in the number of osteoblasts with an optimal dose of 0.3 mg /kg BW, and the extract also increases in bone density seen from an increase in the density of active trabeculae and osteoblasts with an optimal dose of 900mg/kg BW [10,14]. *Cissus quadrangula Salibs* stem extract has the potential as a supplement or drug to help the process of osteogenesis and repair of bone damage [14-15]. This study is conducted to assess the increase in the length growth and the number of cells osteoblasts in the femur of mice after being administered the *Cissus quadrangula Salibs* stem extract dose of 500 ml/Kg BW, 700 mg/Kg BW, and 900 mg/Kg BW, and to know the effective dose which affects the length growth and the number of cells osteoblasts of the Femur male mice strain DDY.

II. Materials and Methods

Research is experimentally laboratory with comparative methods. The object of the study used 24 male mice of DDY strains that have been adapted for 7 days. The grouping of mice consisted of 4 groups with each group consisting of 6 mice. P0 (n=6), negative control group, given only standard feeding, P1 to P3 was the treatment group given *Cissus quadringularis Salibs* stem extract with dose P1 500 mg/Kg BW, P2 700 mg/Kg BW), and P3 900mg/Kg BW.14 This study has obtained ethical approval from the Ethics Committee of Padjadjaran University Bandung Indonesia. *Cissus quadrangula Salibs* stem extract 500 mg/Kg BW, 700 mg/Kb BW, and 900 mg/Kb BW administered orally daily for 30 days, then measured femur length and calculated the number of osteoblast on the Femur metaphysis plate. The data were analyzed using One Way ANOVA and continued with Duncan's post hoc test.

III. Results and Discussion

A. *Effect of Cissus quadrangula Salibs Extract on Male Mice Femur Length*

Research related to the influence of *Cissus quadrangula Salibs* stem extract has been conducted in the animal laboratory of Padjajaran University. The research was conducted from November 2019 to January 2020, in 4 mice groups, each of which consists of 6 mice that have met the criteria of exclusion and inclusion. Results from femur length measurements show that there is a difference in Femur length between the treatment group and the control group, as in Table 1.

TABLE I. Description of Mice Femur Length

Groups	Average Femur Length
P0 : control group	1,23±0,06
P1: Treatmen group dosage 500 mg/kg BW	1,35±0,04
P2: Treatmen group dosage 700 mg/kg BW	1,39±0,09
P3: Treatmen group dosage 900 mg/kg BW	1,54±0,10

Table 1 showed significant differences in the length of Femur Os between the control groups compared to the treatment groups. The control group showed the shortest results, whereas P3 were given *Cissus Quadrangula Salibs* extract dose of 900mg/Kg BW indicating the longest femur. When compared between groups, the length of the femur increases when the dosing increases. This indicates that the larger the dose given, the longer the length of the mice femur. See table 2 below.

TABLE II. One Way Anova of Male Mice Femur Length

	Mean	P value
Between groups	0,097	0,000
Within groups	0,006	

Based on the analysis test using one Way Anova (Table 3.2) obtained a value of p = 0.000 ($p \leq 0.05$), so it can be concluded that there are significant differences between each treatment group. Based on these results, then conducted Duncan tests to find out which groups had significant differences in values (Table 3).

Table 3 showed significant differences between the P0 versus P2 and P3, P1 versus P3, and P2 versus P3, which indicates a value of $p \leq 0.05$. While the group that does not differ significantly is P0 compared to P1, and P1 compared to P2. Administration of *Cissus quadrangula Salibs* stem extract dose of 900 mg/Kg BW gave statistically significant results to increase the length of the male mice femur. The results of this experiment also showed that the larger dosage given, the greater influence of compounds contained in the stem of *Cissus quadrangula Salibs* such as phytoestrogens and calcium, against the length of the male mice femur [10,14-16]. It can be concluded that the extract of *Cissus quadrangula Salibs* stem can increase the length of os Femur mice male strain DDY.

TABLE III. DUNCAN TEST OF MALE MICE FEMUR LENGTH

Variable	*P value*	Interpretation
Group P0 vs P1	0.085	The difference is insignificant
Group P0 vs P2	0.013	Significant differences
Group P0 vs P3	0,000	Significant differences
Group P1 vs P2	0.803	The difference is insignificant
Group P1 vs P3	0.003	Significant differences
Group P2 vs P3	0.019	Significant differences

Description: P0: Negative control

P1: *Cissus quadrangula* extract *500 mg/Kg BW*

P2: *Cissus quadrangula* extract *700 mg/Kg BW*

P3: *Cissus quadrangula* extract *900 mg/Kg BW*

TABLE VI. DOUBLE COMPARISON TEST

Variable	*P value*	Interpretation
Group P0 vs P1	0,069	The difference is insignificant
Group P0 vs P2	0,001	Significant differences
Group P0 vs P3	0,000	Significant differences
Group P1 vs P2	0,137	The difference is insignificant
Group P1 vs P3	0,000	Significant differences
Group P2 vs P3	0,000	Significant differences

Description: P0: Negative control

P1: *Cissus quadrangula* extract *500 mg/Kg BW*

P2: *Cissus quadrangula* extract *700 mg/Kg BW*

P3: *Cissus quadrangula* extract *900 mg/Kg BW*

B. Effect of Extract Cissus quadrangularis Againts Total Osteoblast Male Mice Femur

The results of the administration of *Cissus quadrangula Salibs* stem extract to influence the number of osteoblasts in femur mencit Male Strain DDY showed in Table 4. The number of osteoblasts differed between the treatment group and the control group. The number of osteoblasts in P0 that was only given standard feed showed the least number of osteoblasts at least 73 ± 4.18, while in the P3 group given dose 900 mg/Kg BW showed the most number of osteoblasts that is 102 ± 3.08. Based on histopathological observations, there are differences in osteoblast density from each test group that has been given *Cissus quadrangula Salibs* stem extract. The density of femur mice osteoblast cells in each group can be seen in Figure 3.1. When compared between treatment groups, it can be seen that the number of osteoblasts in the group given Sipatah-broken extract dose 900 mg/Kg BW (P3) appears more closely compared to the dosage group of 500 mg/Kg BW and 700 mg/Kg BW. This indicates that the larger the dose given, the more the osteoblast number in femur.

TABLE IV. THE NUMBER OF OSTEOBLAST IN MALE MICE FEMUR

Groups	Average of Osteoblast
P0 : control group	73 ± 4,18
P1: Treatment group dosage 500 mg/kg BW	80 ± 4,24
P2: Treatment group dosage 700 mg/kg BW	86 ± 4,74
P3: Treatment group dosage 900 mg/kg BW	102 ± 3,08

TABLE V. ONE WAY ANOVA TEST FOR THE NUMBER OF OSTEOBLASTS IN OS. DDY MALE MICE FEMUR

	Mean	*P value*
Between groups	764.583	0,000
Within groups	16.875	

Table 5 shows the value of p=0.000 of (p< 0.05). There are meaningful differences between each treatment group. Duncan's advanced test (Table 3.6) was conducted to find out which group had significantly different values based on the results of the One Way Anova test.

Table 6 shows insignificant differences between the P0 versus P2 and P3 groups, there are significant differences between the P1 versus P3 groups, and there are significant differences between the P2 group versus P3. It is shown from the p≤0.05 value. The group that did not differ significantly was P0 compared to P1 and P1 compared to P2. Based on the data, it is concluded that the P3 group with a dose of 900 mg/KgBB had a significant role in the addition of osteoblast count in the femur. The administration of *Cissus quadrangular Salibs* stem extract at a dose of 900 mg/Kg BW provided statistically significant results to increase the number of osteoblasts in the male mice femur. This experiment also showed that the larger of dosage, the greater influence of compounds contained in *Cissus quadrangular Salibs* stem extracts, such as phytoestrogens and calcium, on the number of femur male mice [10,14-16]. It can be concluded, that plant extract fractures can increase the number of osteoblast cells in femur male mice.

The content of *Cissus quadrangular Salibs* stem extract is beneficial for femur growth. It was seen from the difference in length of the femur os control group with the treatment group. The Growth in bone length is influenced by several factors, such as age, nutrient intake, and hormonal. In this study, age factors can be controlled by choosing mice, that are in their infancy, mice aged 8-12 weeks [17]. Hormonal factors can also be controlled by choosing male mice, to eliminate the suspected estrogen which can affect the increase in bone growth [18,19].

Cissus quadrangula Salibs is a plant known for its ability to treat fractures. The most dominant content of *Cissus quadrangula Salibs* is phytoestrogens and calcium. These two components also play an important role in influencing bone growth, with mechanisms [20-22]:

- Phytoestrogens contained in *Cissus quadrangula Salibs* can increase osteoblast cell activity and reduce osteoclast cell activity by increasing igf-1,c-fos activity which stimulates osteoblast and c-bfa activity and increases osteoprotegerin gene activity which decreases osteoclast activity [23,24].
- The effectiveness of calcium absorption in the intestines is also affected by calcium intake. The lower the calcium content in the food consumed, the more active the intestine will be to perform absorption. As much as

99% extracellular calcium is present in the bone in the form of hydroxyapatite which describes the balance between bone formation and resorption. Calcium can inhibit cytokines IL-1, IL-6, and TNFα so as to decrease osteoclast activity [10,14,15,25].

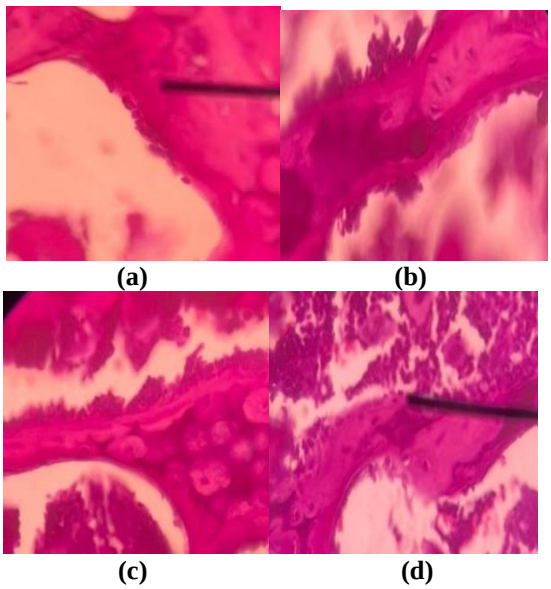

Fig. 1. The arrows show the osteoblasts on Ossa Femur of mice (a) Group P0 (b) Group P1 (c) Group P2 (d) Group P3 at 400x magnification.

Phytoestrogens and calcium content in *Cissus quadrangula Salibs* extract can increase osteoblast activity and decrease bone osteoclast activity, so there is a difference in femur length between the treatment group and the male mice control group. This is supported by the results of a study in 2011 that showed that cissus quadrangula salibs extract has the potential to improve bone condition in female rats so as to prevent osteoporosis in the future. Another study was conducted in 2003 and 2019 to see the effect of cissus quadrangula Salibs extract on mice with osteoporosis, using different species of *Cissus quadrangula Salibs.* As a result, *Cissus quadrangula Salibs* can decrease osteoclast activity so as to prevent more severe bone damage due to osteoporosis [14-16]. Another study in 2014 showed that the administration of *Cissus quadrangula Salibs* extract can increase the proliferation and differentiation of bone marrow mesenchymal stem cells into osteoblast cells [10].

IV. Conclusion

Cissus quadrangula Salibs stem extract can significantly increase bone length and osteoblast cells of the femur at doses of 700 mg/Kg BW and 900 mg/Kg BW. The effective dosage of *Cissus quadrangula Salibs. Stem extract* to increase bone length and the number of osteoblast cells is 900mg/Kg BB.

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