

Efficacy of Randu Honey Supplementation on Callus Formation in Closed Fractures of the Tibia in Wistar Rats

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ABSTRACT

Introduction: Tibial fracture is one of the most common injuries. Randu honey has the potential to speed up fracture healing. This study aims to determine the efficacy of randu honey supplementation on callus formation in closed fractures of the tibia in Wistar rats. **Methods:** This research uses a true experimental design with post test only control group design. A total of 36 Wistar rats were divided into two groups, namely the control group and the treatment group which were given 200g/BW(gram)/day of randu honey. Fractures were made on the rat tibia and callus diameter was measured at 3 weeks after treatment. **Results:** The callus diameter in the treatment group was significantly larger than the control group ($p < 0.05$) in radiological and macroscopic measurements. **Conclusion:** Supplementation with randu honey is effective in the formation of callus in closed fractures of the tibia in Wistar rats.

1. Introduction

Tibial fractures, especially closed fractures, are one of the musculoskeletal injuries frequently encountered in clinical practice. This injury can occur due to various factors, such as traffic accidents, falls, or direct impacts on the lower limbs. Tibial fractures can cause significant pain, impair mobility, and reduce the patient's quality of life. Treatment of tibia fractures generally involves repositioning of the broken bone and stabilization with a cast, internal fixation (pen), or a combination of both. However, the fracture healing process takes a long time and carries the risk of complications, such as infection, malunion (incomplete bone union), and nonunion (failure of the bones to fuse). Based on epidemiological data, tibia fractures are one of the most common types of

fractures, with an incidence of around 20-30 cases per 100,000 people per year. This fracture occurs more often in men than women and generally occurs in young and active age groups.¹⁻³

The impact of tibia fractures is not only limited to pain and impaired mobility but can also have significant economic consequences. The cost of treating and rehabilitating a tibia fracture can reach millions of rupiah, and patients may experience a loss of income during the recovery period. Efforts to speed fracture healing and reduce complications continue to be made by researchers and clinicians. Various methods have been studied, including the use of medications, physical therapy, and electrical stimulation. However, there is still a need for more effective and safer solutions to accelerate fracture

healing. Randu honey has various bioactive properties that are useful for healing wounds and fractures. Randu honey contains flavonoid compounds and phenolic acids, which can help reduce inflammation and swelling around the fracture area. Randu honey has antimicrobial activity, which can help prevent infections in wounds and fracture areas. Randu honey is rich in antioxidants, which can help protect cells from damage caused by free radicals, thereby speeding up the healing process. Randu honey contains several growth factors that can help stimulate the growth of bone cells and tissue around the fracture area.^{4,5} This study aims to determine the efficacy of randu honey supplementation on callus formation in closed fractures of the tibia in Wistar rats.

2. Methods

This research uses a true experimental design with a post-test-only control group design. This design was chosen to evaluate the effect of randu honey supplementation on callus formation in closed fractures of the tibia in Wistar rats. A total of 36 male Wistar rats with a body weight of 200-250 grams and 3 months of age were used in this study. Rats were divided randomly into two groups, namely the control group and the treatment group. The control group was not given randu honey. The treatment group was given 200g/BB(gram)/day randu honey supplementation. Randu honey is given orally using a syringe for 3 weeks.

Fractures were created in rat tibias using modified three-point bending pliers. The fracture is created in the middle of the tibia with sufficient force to produce a complete transverse fracture. The callus diameter was measured at 3 weeks after treatment using two methods: 1. Radiology: The callus diameter was measured on X-rays of the tibia using a micrometer. 2. Macroscopic: Rats were sacrificed, and the tibia was extracted. The callus diameter was measured on a cross-section of the tibia using a micrometer. Data were analyzed using appropriate statistical tests, namely the t-test and Mann-Whitney U test, with p-value <0.05. This research was conducted following applicable research ethics rules. Approval from the ethics commission of the Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia, was obtained before the research began.

3. Results

Table 1 shows that there is a statistically significant difference in the radiological callus diameter before and after treatment in the randu honey group (p=0.001). This shows that giving randu honey is effective in increasing the diameter of radiological callus in Wistar rats with tibia fractures. In the control group, the difference in radiological callus diameter before and after treatment was not statistically significant (p>0.05). This shows that there is no treatment effect in the control group.

Table 1. Comparison of differences in radiological callus diameter before and after treatment.

Group	Mean ± SD	P value
Randu honey	1,935 ± 0,460	0,001*
Control	0,542 ± 0,520	

Independent T-Test, *p<0,05.

Table 2 shows that there is a statistically significant difference in the macroscopic callus diameter after treatment between the randu honey group and the control group (p=0.001). This shows that giving randu honey is effective in increasing the macroscopic callus diameter in Wistar rats with tibia

fractures. The macroscopic callus diameter in the randu honey group (6.081 ± 1.070 mm) was significantly larger than the control group (4.486 ± 0.527 mm). This shows that giving randu honey can accelerate callus formation in Wistar rats with tibia fractures.

Table 2. Comparison of macroscopic callus diameters after treatment.

Group	Mean ± SD	Median (min-max)	P value
Randu honey	6,081 ± 1,070	6,125 (4,35-8,30)	0,001*
Control	4,486 ± 0,527	4,40 (3,70-5,60)	

Independent T-Test, *p<0,05.

Table 3 shows that there is a significant positive correlation between radiological diameter and macroscopic diameter (r=0.716, p=0.000). This shows that there is a strong relationship between these two variables. The r value of 0.716 indicates that there is

a fairly strong relationship between radiological diameter and macroscopic diameter. This means that the larger the radiological diameter, the larger the macroscopic diameter.

Table 3. Correlation between radiological diameter and macroscopic diameter.

Variable	r	P value
Radiological diameter	0,716	0,000*

Spearman Rho's Test, * p < 0.05.

4. Discussion

The results of the study showed that giving randu honey supplementation was effective in increasing the diameter of radiological callus in Wistar rats with tibia fractures. This is in line with several previous studies that have shown the positive effect of honey on the healing process of wounds and fractures. The effectiveness of randu honey in increasing radiological callus diameter can be explained through several biological mechanisms. Several previous studies have shown the positive effect of honey on the healing process of wounds and fractures. Studies in rats with incision wounds show that topical administration of honey can speed up the wound-healing process and increase the formation of granulation tissue. Research on rabbits with tibia fractures shows that oral administration of honey can speed up the fracture-healing process and increase bone strength.⁶⁻⁸

Randu honey contains various bioactive compounds, including flavonoids and phenolic acids. These compounds have anti-inflammatory effects that can help reduce inflammation and swelling around the fracture area. Flavonoid compounds have the ability to inhibit the activity of enzymes involved in the inflammatory process, such as cyclooxygenase (COX) and lipoxygenase (LOX). Inhibition of these enzymes can reduce the production of prostaglandins and

leukotrienes, which are inflammatory mediators. Phenolic acid compounds have antioxidant properties that can help fight free radicals. Free radicals can cause cell and tissue damage, which can worsen inflammation. Phenolic acids can also inhibit the activity of inflammatory enzymes and increase the production of anti-inflammatory compounds. Inflammation and swelling around the fracture area can hinder the healing process. Inflammation can cause pain and tenderness around the fracture area. Reducing inflammation can help reduce aches and pains so patients are more comfortable and can move more easily. Prolonged inflammation can hinder the fracture healing process. Reducing inflammation can help speed up the healing process and callus formation. Uncontrolled inflammation can increase the risk of complications, such as infection and delayed union/nonunion of fractures. Reducing inflammation can help prevent these complications. Several studies have shown honey's anti-inflammatory effects. In vitro studies show that honey can inhibit the activity of COX and LOX enzymes, as well as the production of prostaglandins and leukotrienes. Animal studies show that honey administration can reduce inflammation and swelling in various inflammatory disease models, including arthritis and sepsis.⁹⁻¹¹

Randu honey has antimicrobial activity, which can help prevent infections in wounds and fracture areas. Infection can hinder the healing process and callus formation. Randu honey produces hydrogen peroxide naturally through the enzyme glucose oxidase. Hydrogen peroxide is an antimicrobial agent that can kill various types of bacteria, fungi, and viruses. Randu honey has a low water content, around 17-20%. This creates a hypertonic environment that can draw water out of the microorganism's cells, causing dehydration and cell death. Randu honey has a low pH, around 3.2-4.5. This acidity can inhibit the growth of microorganisms that cannot live in an acidic environment. Randu honey contains several natural antimicrobial compounds, such as flavonoids, phenolic acids, and methylglyoxal. This compound can damage the cell membrane of microorganisms and inhibit their growth. Randu honey contains prebiotics, which are substances that can help the growth of good bacteria in the intestines. These good bacteria can help fight bad bacteria that can cause infections. The antimicrobial activity of randu honey can help prevent infections in wounds and fracture areas. Infection can hinder the healing process and callus formation. Randu honey can help speed up the fracture healing process by preventing infection. A study on rats with burns showed that topical application of honey could reduce the number of bacteria in the wound area and speed up the healing process. A study in patients with open bone fractures showed that using honey as a wound dressing may help prevent infection and increase healing rates.¹²⁻¹⁴

Randu honey is rich in antioxidants, such as flavonoids, phenolic acids, and enzymes. This antioxidant can help protect cells from damage caused by free radicals. Free radicals are unstable molecules and can cause cell damage through the oxidation process. Cell damage caused by free radicals can inhibit the healing process and callus formation. Free radicals can damage bone cells, such as osteoblasts and osteocytes, which play an important role in bone formation. The antioxidants in randu honey can help protect these bone cells from damage, thereby

increasing their activity and accelerating callus formation. Angiogenesis is the process of forming new blood vessels. These new blood vessels are important for bringing oxygen and nutrients to the fracture area, which helps speed up the healing process. The antioxidants in randu honey can help increase angiogenesis, thereby accelerating callus formation. Chronic inflammation can hinder the healing process and callus formation. The antioxidants in randu honey have anti-inflammatory properties which can help reduce inflammation in the fracture area, thereby speeding up the healing process. Collagen is an important protein that plays a role in the formation of connective tissue, including bones. The antioxidants in randu honey can help increase collagen production, thereby increasing the strength and density of the callus. Several studies have shown that antioxidants can help improve the healing process and callus formation. Studies in rats with tibia fractures show that administration of the antioxidants vitamins C and E can speed up the fracture healing process and increase bone strength. Research in humans with bone fractures shows that administering antioxidant supplements can help reduce pain and speed the healing process.^{15,16}

Randu honey contains several growth factors that are useful for accelerating callus formation in fractures. Insulin-like growth factor-1 (IGF-1) plays an important role in the proliferation and differentiation of osteoblast cells, namely the cells responsible for bone formation. Transforming growth factor-beta (TGF-beta) plays a role in the regulation of collagen and proteoglycan synthesis, which is an important component of the bone matrix. Platelet-derived growth factor (PDGF) plays a role in stimulating the proliferation and migration of osteoblast cells and other cells involved in the bone healing process. Epidermal growth factor (EGF) plays a role in stimulating the proliferation and differentiation of keratinized cells, which is important for wound closure and protecting the fracture area. The combination of growth factors and other bioactive compounds in randu honey can help speed up the fracture healing

process and increase the diameter of the callus. Research on rats with tibia fractures shows that oral administration of honey can improve levels of IGF-1 and TGF-beta in the fracture area, which contributes to accelerated callus formation. Research on human osteoblast cells shows that randu honey can increase the proliferation and differentiation of osteoblast cells, which are important for bone formation.¹⁴⁻¹⁷

5. Conclusion

There was an increase in the diameter of the radiological callus after giving randu honey. Giving randu honey supplementation is effective in the formation of callus rats *Rattus norvegicus* Wistar strain.

6. References

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