The Influence of Honey Consumption on Tibia Bone Fracture Healing in Wistar Rats: A Comprehensive Study

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Abstract

Introduction: Fracture healing is a complex biological process crucial for restoring bone integrity and function. Honey has garnered research interest in the context of bone fracture healing due to its potential to expedite the healing process and strengthen bone structures. Honey also exhibits significant anti-inflammatory activity and influences the differentiation of bone cells and has found to protect bones by reducing resorption and increasing calcium absorption.

Aim: Analyzing histopathological differences in tibia bone fractures in several groups of Wistar rats after administration of Nusantara honey, Manuka honey, and Wild honey.

Methods: The experimental design included inducing tibia fractures in Wistar rats and administering honey orally as a potential therapeutic intervention. Utilizing a posttest only control group design, rats meeting the eligibility criteria were randomly assigned into four groups: one control and three treatment groups. Following a 15-day follow-up period, euthanasia was performed, and histopathological assessment of tibial bone tissue was conducted by an expert anatomical pathologist using the Huo et al. classification system. Data analysis was subsequently carried out to evaluate the experimental outcomes.

Results: The histopathological classification assessment revealed that honey exhibited superior effects compared to control group. Also, manuka honey exhibited superior effects compared to other types of honey in promoting the healing of tibial bones in experimental rats in promoting the healing of tibial bones in experimental rats.These findings suggest the promising role of honey in enhancing bone fracture healing

Keywords: honey, tibia bone fracture, Wistar rats, healing process

1. Introduction

Bone fractures represent a major public health concern worldwide, with substantial morbidity, mortality, and economic implications, especially in regions with limited healthcare resources. Fracture healing is a dynamic process involving various cellular and molecular factors, and disruptions in this process can lead to delayed or non-union fractures. Traditional fracture management strategies often involve surgical intervention and immobilization, but adjunctive therapies are continually being explored to enhance healing outcomes and reduce complications. Honey, with its rich composition of bioactive compounds, has emerged as a promising candidate for promoting fracture healing due to its multifaceted pharmacological properties.[1,2,3]

The physiological process of fracture healing encompasses inflammation, repair, and remodeling phases, orchestrated by a myriad of cellular and molecular signals. Osteoblasts and osteoclasts play pivotal roles in bone formation and resorption, respectively, while growth factors, cytokines, and matrix proteins regulate their activities. Disruption in the delicate balance between bone resorption and formation can impair fracture healing, emphasizing the importance of understanding the underlying mechanisms for developing effective therapeutic interventions.[2,4]

Honey, a natural product derived from bees, has been used for centuries in traditional medicine for its diverse medicinal properties. Recent research has elucidated its potential application in fracture healing, attributed

to its antioxidant, anti-inflammatory, and immunomodulatory effects. Polyphenols, flavonoids, and other bioactive compounds present in honey have been shown to modulate inflammatory cytokines, promote osteoblast differentiation, and inhibit osteoclast activity, thereby facilitating the bone healing process. Clinical studies in animal models have demonstrated promising outcomes with honey supplementation, indicating its potential as an adjunctive therapy for fracture management.[3,4,5]

Manuka honey, derived from the nectar of the Manuka tree (Leptospermum scoparium) by the Apis mellifera honeybee, is renowned for its distinct composition and therapeutic properties. Manuka honey exhibits a complex chemical composition comprising carbohydrates, minerals, proteins, fatty acids, phenolic compounds, and flavonoids. Notably, it contains high levels of methylglyoxal (MGO), derived from dihydroxyacetone (DHA) present in Manuka tree nectar, which correlates strongly with its antibacterial activity. Leptosperin, another unique compound found in Manuka honey, serves as a marker for its authenticity. Despite sharing some constituents with other types of honey, Manuka honey's unique chemical profile contributes to its distinct therapeutic properties.[6]

Forest or wild honey consists primarily of water, carbohydrates, proteins, vitamins, minerals, and enzymes. Its carbohydrate content is notably high, predominantly comprising fructose and glucose, while proteins, amino acids, vitamins (such as B complex vitamins and vitamin C), and minerals (including sodium, calcium, magnesium, iron, phosphorus, and potassium) contribute to its nutritional profile. Enzymes present in forest honey, such as diastase, invertase, glucose oxidase, peroxidase, and lipase, play crucial roles in carbohydrate metabolism and antioxidant defense mechanisms.[7]

Randu or Ceiba honey, classified as a monofloral variety originating from Ceiba pentandra flowers, presents distinctive physical traits and biochemical properties. The antioxidant capacity of Ceiba honey stems from its phenolic compounds, particularly flavonoids, which inhibit microbial and fungal growth. Phenolic compounds serve as potent antioxidants, combating oxidative damage induced by reactive oxygen species such as oxygen, hydroxyl radicals, and lipid peroxides. Ceiba honey demonstrates superior antibacterial activity compared to other honey varieties, including rambutan, forest, and longan honey, attributed to its higher phenolic and flavonoid content. By scavenging free radicals and inhibiting oxidative reactions, Ceiba honey contributes to overall health and well-being, offering potential therapeutic benefits in combating various oxidative stress-related disorders.[4,8]

There is a study investigating the fracture healing properties of honey and hydroxyapatite in rats, significantly better fracture healing was observed through enhanced bone formation, union, and bone remodeling on radiographic scores in rats treated with honey autograft compared to other treatment groups in the second week.[3,9]

In another research, the bone healing effect of honey was examined by Hajizadeh et al. using a mandibular bone defect healing model in rats. A 2 × 2 mm defect was created by extraoral incision at the angle of the mandible. In the experimental group, the defect was filled with sterile honey from the brand Medihoney (Derma Sciences Inc., Princeton, USA). The defects were left unfilled in the control group. After two weeks, five samples from the experimental group were in the mineralization phase, while all samples from the control group were in the vascularization phase. After four weeks, defects were filled in four samples from the experimental group, whereas all samples from the control group were in the mineralization phase. Histomorphometric assessment revealed that new bone formation in the experimental group was significantly superior to the control group after two and four weeks. This study demonstrates that honey can enhance and expedite bone repair in small mandibular defects in rats.[10,11]

Due to the antioxidant, anti-inflammatory, immunomodulatory, and osteoblastogenesis-promoting effects exerted by types of honey, which play significant roles in bone fracture healing mechanisms through osteoblast regulation, honey is expected to serve as an effective agent in fracture healing. Therefore, researchers are interested in investigating the influence of honey on the healing of tibia bone fractures in Wistar rats.[12]

2. Methods

This experimental study aimed to investigate the influence of three different types of honey on tibia bone fracture healing in Wistar rats. The study was conducted at the Laboratory of Mathematics and Natural Sciences, Universitas Sumatera Utara, over a period of two months (December 2023 - January 2024). The population consisted of Wistar strain rats obtained from the same laboratory. Pure honey without any additional ingredients was used in the experiment, and the rats were provided with standard feed. Sample size calculation using the

Federer formula determined that each treatment group required a minimum of 24 rats. To account for potential deaths or illnesses during the study, an additional 20% of rats were added to each experimental group, resulting in a total sample size of 28 rats per group.

Inclusion criteria included healthy and active male Wistar rats aged approximately 9-12 weeks, weighing around 150-200 grams, with clear eyes, nose, mouth, and anus. Exclusion criteria comprised rats that died or became ill during the study and rats with pre-existing fractures.

All experimental rats meeting the study criteria were housed in clean cages with ad libitum access to food and water. All procedures were conducted by the same operator to maintain consistency. Randomization was performed using a coin toss, and the rats were allocated into four groups: Group A served as the control, while Groups B, C, and D were designated as the treatment groups.

To induce fractures, fractures were created in the tibia bone of the rats. The rats were anesthetized using ketamine and then immobilized on the operating table. An incision was made near the tibial tuberosity, followed by the creation of a tibial fracture. The fracture was induced using a Gigli wire to create a transverse fracture in the medial to proximal third of the tibia. Subsequently, repair and closure were performed as thoroughly as possible.

All rats were then provided with their respective treatment diets: Group A (control) received normal food and water, Group B received Nusantara honey, Group C received Manuka honey, and Group D received wild honey. The honey was administered orally three times a day after meals at a dose of 2 g/kg for 15 days. Evaluation was conducted at the end of the follow-up period.

At the end of the follow-up period, euthanasia was performed on the rats using CO2 asphyxiation followed by cervical dislocation. After euthanasia, tibia bone tissue samples were collected, fractured, and subjected to histopathological examination. Histopathological examination was conducted using the scoring system developed by Huo et al. in 1991 in which divided into 10 scoring: (Score 1) Fibrous tissue; (Score 2) A large amount of fibrous tissue and a small amount of cartilage tissue; (Score 3) Equal ratio of fibrous tissue and cartilage tissue; (Score 4) A large amount of cartilage tissue and a small amount of fibrous tissue; (Score 5) Cartilage tissue; (Score 6) A large amount of cartilage tissue and a small amount of immature bone tissue, (Score 7) Equal ratio of cartilage tissue and immature bone tissue; (Score 8) A large amount of immature bone tissue and a small amount of cartilage tissue; (Score 9) Fracture healing with immature bone; (Score 10) Fracture healing with mature bone.

Data were collected directly by the researchers. Normality tests were conducted using the Shapiro-Wilk test, considering the sample size < 50. To analyze the differences in clinical and histopathological findings, ANOVA would be used if the data were normally distributed, or non-parametric data analysis with the Kruskal-Wallis test if the data were not normally distributed. If both results were significant, post-hoc Bonferroni tests for ANOVA or Mann-Whitney tests for Kruskal-Wallis would be conducted to determine differences between groups. For the analysis of histological classification variables based on intervention type, categorical-categorical measurement scales with a 2xk table using the Kolmogorov-Smirnov test would be applied. A significance level of $p < 0.05$ was considered statistically significant. Statistical analysis was performed using Statistical Product and Service Solutions (SPSS) version 11.0 for Windows.

3. Results

In this study, an experimental method using experimental animals (rats) and employing a posttest only control group was utilized. The total sample size for experimentation comprises 28 rats, which were divided into 4 groups as follows:

Group A: Consisting of 7 rats with tibia fractures, but not subjected to any additional treatment (control group).
Group B: Comprising 7 rats with tibia fractures, administered with consumption of Nusantara honey throughout the observation period.
Group C: Including 7 rats with tibia fractures, given consumption of Manuka honey throughout the observation period.
Group D: Involving 7 rats with tibia fractures, provided with consumption of wild honey throughout the observation period.

At the end of the observation period, an evaluation will be conducted to assess the effects of the treatments on the healing of tibia bone fractures in each group of rats.

Descriptively, histopathological examination was conducted to identify the number of osteoblasts and histopathological features according to the classification by Huo et al. In the study, osteoblasts were observed post-treatment in the histopathological images of the control group rats. However, subjectively, this appeared to be less prominent compared to the Manuka honey, wild honey, or Nusantara honey groups.

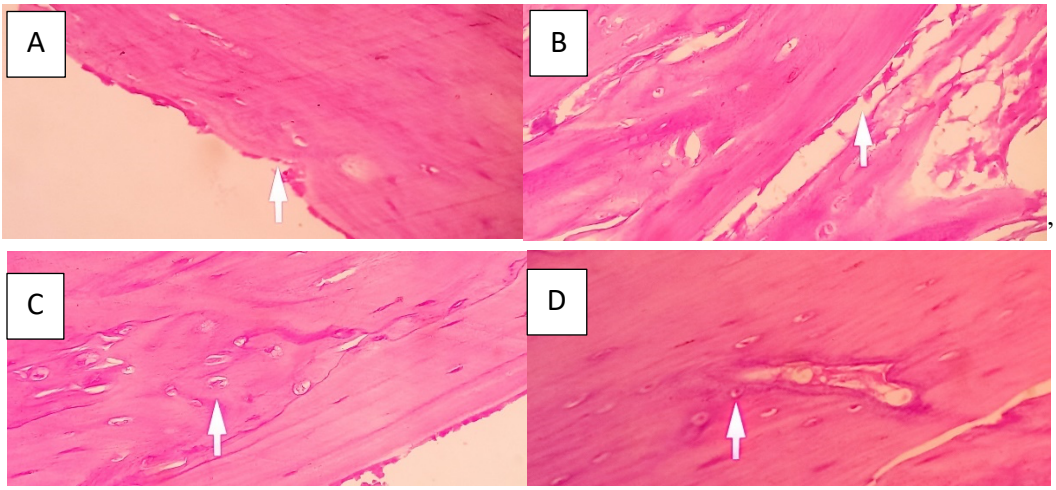

Figure 4.1 Histopathological images of rats post-treatment and follow-up under a microscope at 10x40 magnification. (a) Control, (b) Nusantara Honey, (c) Manuka Honey, (d) Wild Honey. Arrows indicate osteoblasts.

In the histopathological classification assessment, Manuka honey provided a superior effect compared to other honey types and the control group. This is illustrated in Table 4.2.

Table 4.2 Histopathological Classification Overview Post-Treatment

Histopathological Classification	Control	Nusantara Honey	Manuka Honey	Wild Honey	P value*
I	-	-	-	-	<0.001
II	3 (42,9%)	-	-	-	
III	-	1 (14,3%)	-	1 (14,3%)	
IV	3 (42,9%)	2 (28,6%)	-	-	
V	1 (14,3%)	3 (42,9%)	-	4 (57,1%)	
VI	-	1 (14,3%)	1 (14,3%)	1 (14,3%)	
VII	-	-	1 (14,3%)	1 (14,3%)	
VIII	-	-	4 (57,1%)	-	
IX	-	-	1 (14,3%)	-	
X	-	-	-	-	

*) Kolmogorov-Smirnov Test

To visualize the data, the data is presented in Figure 4.2

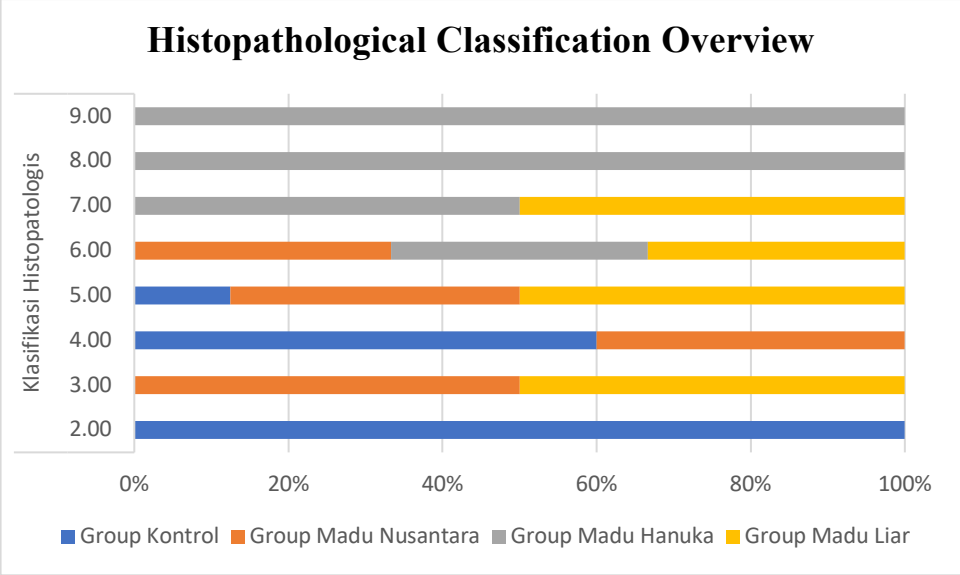

Figure 4.2 Histopathological Classification Overview

In this study, the mean number of osteoblasts per group was analyzed. The mean number of osteoblasts in the control group was 9.8 ± 2.09 compared to 14.42 ± 1.43 in the Nusantara honey group, 27.74 ± 4.7 in the Manuka honey group, and 14.4 ± 3.68 in the wild honey group. Normality test analysis indicated that the data were normally distributed. ANOVA test revealed significant differences among the groups with post-hoc test results presented in Table 4.1.

Groups	Osteoblast mean number	P Value*	Post-hoc Bonferonni Test		
			Nusantara Honey	Manuka Honey	Wild Honey
Control	9,8 ± 2,09	<0.001	0,088	<0,001	0,091
Nusantara Honey	14,42 ± 1,43			<0,001	1
Manuka Honey	27,74 ± 4,7				<0,001
Wild Honey	14,4 ± 3,68				

*) ANOVA Test

To visualize the data, an assessment was conducted with Figure 4.2, which illustrates the superiority of Manuka honey compared to other treatments.

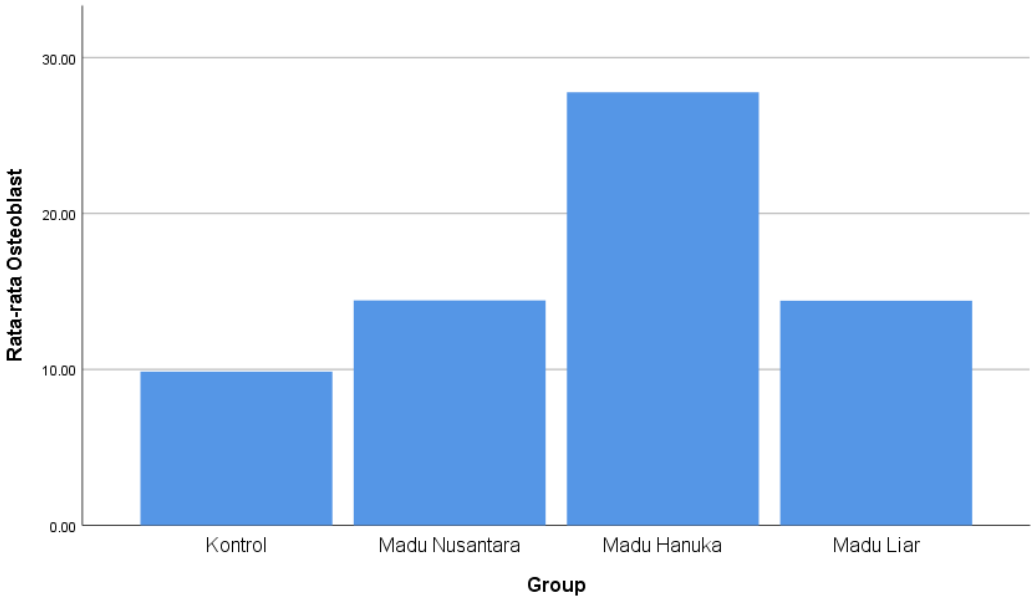

Figure 4.2: Mean Osteoblast Count Post-Treatment

4. Discussion

A study was conducted to assess the effect of honey on the healing of tibial bone fractures. The bone healing process aims to restore tissue to its original physical and mechanical properties and is influenced by various systemic and local factors; healing occurs in three distinct yet overlapping phases. During the bone healing process, several recovery phases facilitate the proliferation and protection of the area surrounding fractures and dislocations. The duration of the process depends on the extent of the injury, with a common timeframe given as 2 or 3 weeks for most upper body fractures and 4 weeks for lower body fractures. The healing process is primarily determined by the periosteum, which serves as a source of precursor cells that develop into chondroblasts and osteoblasts crucial for bone healing. Bone marrow, endosteum, small blood vessels, and fibroblasts are other sources of precursor cells.[2]

In a study investigating the healing properties of honey and hydroxyapatite on rats, significantly better fracture healing was observed through bone formation, union, and bone remodeling, as evidenced by radiographic scores in rats given honey autografts compared to other treatment groups in the second week. Rats treated with honey alone exhibited the poorest healing based on radiographic scores during the treatment period. Histopathological investigation revealed that the group treated with hydroxyapatite alone showed the poorest bone marrow formation compared to all other treatment groups. Therefore, honey and hydroxyapatite together appear to provide better healing effects on bone damage compared to using them separately.[3,13]

The bone healing effect of honey was also investigated by Hajizadeh et al. using a mandibular bone defect healing model in rats. A 2 × 2 mm defect was created with an extraoral incision at the mandibular angle. In the experimental group, the defect was filled with sterilized honey with the brand Medihoney (Derma Sciences Inc., Princeton, USA). The defect was left unfilled in the control group. After two weeks, five samples from the experimental group were in the mineralization phase, while all samples from the control group were in the vascularization phase. After four weeks, the defects were filled in four samples from the experimental group, while all samples from the control group were in the mineralization phase. Histomorphometric assessment showed that new bone formation in the experimental group was significantly better than the control group after two and four weeks. This study demonstrates that honey can enhance and accelerate bone repair in small mandibular defects in rats.[3,14]

Grayanotoxin (GTX) is a toxin found in the flowers of plant species such as Rhododendron and Kalmia. GTX is present in rhododendron pollen and can also be found in honey produced from these plants. Honey containing this toxin is locally known as "mad honey" and is used for alternative therapy. In a study comparing the effects of honey containing GTX at 80 mg/kg/day, normal honey at 80 mg/kg/day, and propolis at 200 mg/kg/day on fracture healing, Sahin et al. showed that honey and propolis containing GTX accelerated the healing of artificially induced transverse fractures over 30 days. GTX and propolis may have similar therapeutic

effects in fracture healing. In a study by Hasib et al., administration of honey (1 g/kg, 2 g/kg, and 4 g/kg for 2 weeks) had beneficial effects on osteoporotic fracture healing in rat femurs by increasing osteoblastogenesis. The pro-osteoblastic effect of honey was evidenced by increased serum alkaline phosphatase levels. Some phenolic compounds in honey have been reported to contribute to the bone-protective effects of honey. Honey contains polyphenols with antioxidant potential, which can enhance the differentiation of mesenchymal cells into osteoblasts. It also plays a role in signaling pathways, such as Wnt and BMP, thereby promoting the differentiation of mesenchymal cells into osteoblasts. Flavanols, such as quercetin and kaempferol, can affect bone resorption by directly inducing osteoclast apoptosis, thereby reducing the number and resorption of bones. Flavanols reduce intracellular ROS in osteoclasts and interact with estrogen receptors in cells. In parallel, Trivedi et al. also found that kaempferol improves osteoblast function, thus preventing bone loss due to ovariectomy. Gluconic acid, a major component of honey, can increase calcium absorption in bones, thus maintaining bone mass and preventing osteoporosis. Gluconic acid is the main organic component in honey produced through the enzymatic reaction of glucose oxidase.[10,11]

Bone fracture healing is a complex and dynamic process regulated by various cellular elements and cytokines in which osteoclasts and osteoblasts are two important cell types. Osteoclasts are responsible for resorbing and remodeling cartilaginous bone during fracture healing by secreting acid and proteinases, while osteoblasts form new bone. Increasing osteoclastogenesis accelerates cartilaginous bone resorption, and increasing osteoblastogenesis during fracture healing promotes bone union, whereas inhibiting osteoclast or osteoblast differentiation is reported to delay bone healing. Osteoclastogenesis and osteoblastogenesis are regulated by many factors, with calcium signaling being one of the important factors.[15]

The limitation of this study is the use of animal studies. Animal studies are early preclinical studies and cannot yet provide strong evidence. Direct studies on humans are needed to confirm the effect of honey administration on tibial fracture healing.

5. Conclusion

There were differences in the mean osteoblast count in the healing of tibial bone fractures in experimental animals based on the type of honey administered. The mean number of osteoblasts in the control group was 9.8 ± 2.09 compared to 14.42 ± 1.43 in the Nusantara honey group, 27.74 ± 4.7 in the Manuka honey group, and 14.4 ± 3.68 in the wild honey group.

There were differences in histopathological classification in the healing of tibial bone fractures in experimental animals based on the type of honey administered, where Manuka honey provided a better effect compared to other honey types and the control group.

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