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Research Article

EFFECT OF MANGOSTEEN PEEL EXTRACT ON BONE FRACTURE HEALING

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ABSTRACT

Objective: Healing of bone fractures is mediated through antioxidants; however, increased free radical levels at the site of fracture inhibit bone healing. Mangosteen peel has antioxidant, anti-inflammatory, antitumor, antiviral, antibacterial, antifungal, antihistaminic, antimalarial, and other beneficial properties as it contains many active substances such as xanthones, anthocyanins, phenols, and tannins. In this study, we aimed to determine the effects of mangosteen peel extract on bone fracture healing.

Methods: We used six mice with left and right femoral fractures (12 femurs). Mangosteen peel extract was applied to the left femurs of the six mice at two dosages (20 and 40 mg/kg; three femurs each) on days 2, 4, and 6 after fracture; saline was injected into the right femurs of all six mice. On day 7, all the animals were sacrificed, and femur defect diameter was evaluated using dental digital radiography.

Results: The femoral defect diameter in mice treated with 40 mg/kg dose of mangosteen peel extract was less than that in mice treated with saline, although the difference was not significant.

Conclusion: Application of a 40 mg/kg dose of mangosteen peel extract promotes bone fracture healing.

Keywords: Bone fracture healing, Dental digital radiography, Mangosteen peel extract.

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INTRODUCTION

Mangosteen fruit (*Garcinia mangostana* Linn.) comes from tropical forest areas in Southeast Asia and can be easily found in Indonesia. The mangosteen pericarp has long been used in Southeast Asia as a traditional medicine for treating stomatitis, dysentery, cystitis, diarrhea, gonorrhea, and exim. Current research on mangosteen peels has shown that they contain bioactive substances such as xanthones, anthocyanins, phenols, tannins, and other derivatives. Several of these bioactive compounds and their derivatives reportedly have a significant pharmacological activity such as antioxidant, antihistaminic, anti-inflammatory, antibacterial [1], and anticancer [1,2] properties.

Fracture is the discontinuity of bone due to trauma or a continuous and pathologic force. Bone fracture cases are considered a serious problem in Indonesia. Reportedly, the total number of bone fracture cases in Indonesia is more or less 1.3 million cases every year, which is highest among the Southeast Asian countries. A survey showed that the prevalence of lower extremity fractures caused by accidents was around 46.2%; these fractures reportedly led to mortality (25%), physical disability (45%), and depression and psychological distress (15%), and only 10% cases were able to heal [3].

A fractured bone normally has the ability to heal itself. According to Altizer (2002) and Robert (2009), bone healing comprises five phases. (1) Hematoma phase (1–3 d after fracture); during this phase, blood coagulation, vessel dilation, debris formation, and exudation of fibrin-contained plasma occurs. (2) Cartilage formation phase (3 d–2 w): During this phase, acute inflammation, formation of fibrocartilage, and periosteum elevation occur. (3) Callus formation phase (2–6 w): During this phase, medulla callus, bone continuity until the formation of trabecular or cancellous bone that replaces the callus. (5) Consolidation-remodeling phase (6 w–1 y): During this phase, the bone shape or function alters so as to enable the bone to function best [4].

In the hematoma phase, the immune system invades the fracture area, causing inflammation that initiates healing and formation of granulation tissue [5]. However, neutrophil activity within the fracture area produces high levels of free radicals, which have been shown to inhibit bone healing by damaging cell membranes and triggering cell lysis. Antioxidants are needed to inhibit such free radical activity [6-8]. In the present study, we investigated the antioxidant effects of mangosteen peel extracts on bone fracture healing in mice.

METHODS

Animals and bone defect generation

Dissection instruments were sterilized using betadine solution or 70% alcohol. Mice (n=7) were anesthetized using an intraperitoneal injection containing 80 mg/kg ketamine and 10 mg/kg xylazine. The mice thighs were shaved to ease the dissection procedure.

The mice thigh skin was smeared with betadine or 70% alcohol. The femur was then exposed using a scalpel and no. 12 surgical blades. Defects were made in each femur with a diamond round bur using a low-speed micromotor until reaching the depth of the bur head. Defects were made in both the left and right femurs of all the animals using the same technique. One mouse was sacrificed by directly placing it inside an ether-filled container, and the femurs from both the legs were used as the defect standard measurement (control). One ear of each of the other six mice was marked by cutting a small notch out of it using surgical scissors. Simple interrupted suturing was performed using nonresorbable silk sutures to facilitate the easy reopening of the incision if a femur sample was needed for further testing. The suturing was performed on muscle and skin tissues using the same suture pattern on each leg. During suturing, the needle can be held only using a needle holder in the dominant hand to prevent a possible needle punctured wound to the operator's hand. The muscle and skin tissues could be held using tissue forceps in the non-dominant hand. After suturing, the area was again cleaned using betadine or 70% alcohol. Mice were returned to their cages and monitored before mangosteen peel extract application.

Drug treatments

Mangosteen peel extract was applied, and saline solution was administered to the mice through an intraperitoneal injection (0.2 mL saline/injection) in a localized position on days 2, 4, and 6 after making the defects. Three mice received 20 mg/kg mangosteen peel extract, and three received 40 mg/kg extract in the left leg, and all six mice received a saline solution in the right leg. Mice were anesthetized using a centrifuge tube filled with cotton and ether before each injection. On day 7 after fracture, mice were euthanized by directly placing them inside an ether-filled container, and femur samples were collected. The samples were stored in centrifuge tubes filled with 70% alcohol until radiographic image processing.

Radiography

Stored femur samples were secured using sellotape and then glued before being exposed to X-ray using dental digital radiography. Each radiograph contained a pair of femur samples from the same mouse. After images were digitized, the measurement scale was calibrated, and defect diameters were measured.

Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA) and a *post hoc* test. Defect diameters were measured using Digora for Windows at the end of the treatment period and mean defect diameters after extract application and saline administration were evaluated using SPSS.

RESULTS

Fig. 1 shows representative radiographic images of control and saline-treated mouse fracture defects.

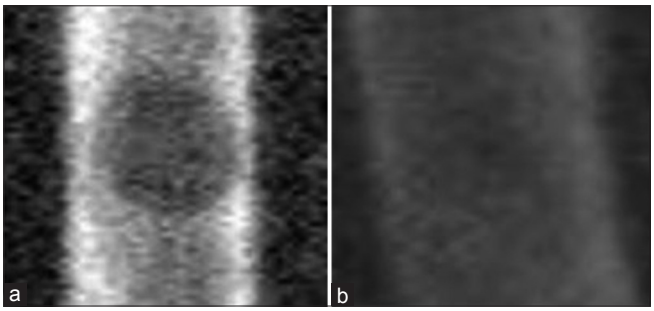

Fig. 1: Radiographic images of mouse femurs (a) control femur (untreated) (b) saline-treated mouse femur after 7 d

The mean defect diameter in the mice 7 days after fracture was 1.17 mm for the femurs with 20 mg/kg dosage of extract, 1.20 mm for the femurs with the 40 mg/kg dosage, and 1.0678 mm for the saline-treated femurs, indicating greater healing with saline. However, both ANOVA and *post hoc* test results showed that there was no significant difference in the defect diameter among the three treatment groups ($p>0.05$).

DISCUSSION

After fracture, the bone heals itself in five main phases. During the hematoma phase, phagocytic cells, such as neutrophils, enter the fracture area, and generate free radicals like nitric oxide, which modulate fracture healing [4,7]. However, these free radicals have also been shown to inhibit or even damage the bone healing process [7]. A previous study showed that xanthone derivatives in mangosteen peel extract could prevent free radical damage, which is similar to the role played by Vitamin C [9]. Therefore, the present study investigated the antioxidant effects of mangosteen peel extract on bone fracture defects in the femur of mice.

Our study results demonstrated that administration of mangosteen peel extract in dosages of 20 mg/kg and 40 mg/kg inhibited bone healing, though not significantly, as defect diameters in femurs treated with both the dosages were higher than that in saline-treated femurs (Fig. 2). This result is in line with a study by Diwan *et al*., who showed that free radicals, such as nitric oxide, are actually necessary in the bone healing process [10]. Thus, local treatment with antioxidants present was detrimental to bone repair.

The 20 mg/kg dosage of mangosteen extract was set as baseline based on the research by Shibata *et al*. who stated that this dosage is pharmacologically effective against cancer yet nontoxic [2]. In another research on anticancer agents, it was reported that mangosteen peel extract dosage of not only 20 mg/kg but also 40 mg/kg showed a significant effect [11]. Furthermore, Kosem *et al*. showed a mangosteen peel extract dosage of ≤50 mg/kg to be nontoxic [12]. Thus, the above dosages were chosen in the present study.

The mangosteen pericarp is known to have strong antioxidant properties and has been predicted to accelerate the healing process [13,14]. Previously, Gokturk *et al.* showed that defects created in mouse femurs treated only with saline healed within 22 days, as observed radiographically [7]. We did not wait for 22 days based on the assumption that it would not be easy to radiographically evaluate the healed defect (especially if it gets closed). Thus, the mice were kept alive only for 7 days so that the mice femur bone with the defect could be clearly evaluated radiographically.

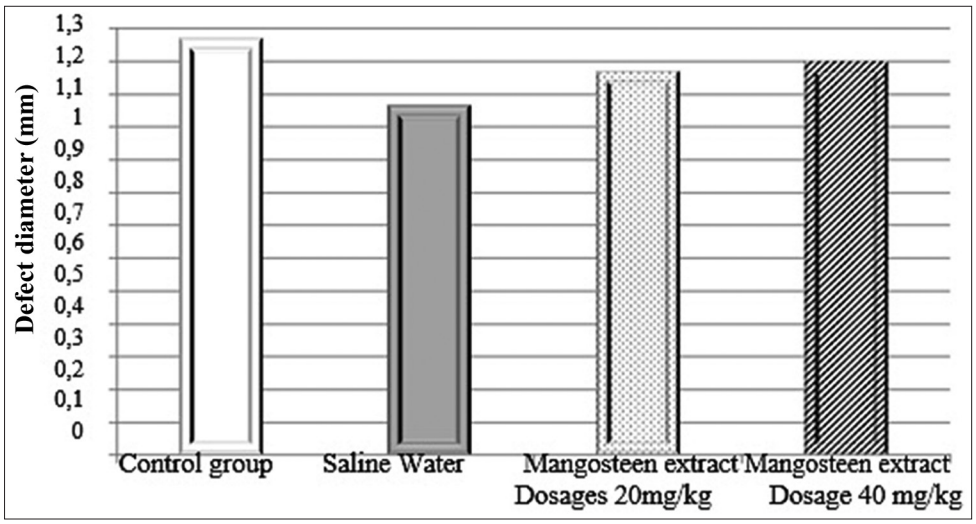

Fig. 2: Defect diameter of Femur after treatment

The radiographic evaluation performed in this study was similar to that performed by Gokturk *et al*. who used dental digital radiography [7] was able to show that administration of mangosteen peel extract actually slowed the healing process. It is possible that extended treatment may show more significant results. This tool offers the advantage of enabling radiographic image manipulation, such as setting the contrast and magnification. Furthermore, the required measurements can be appropriately taken and image acquisition time is short. However, this tool also has certain disadvantages. As the acquired image is two-dimensional, the defect diameter cannot be determined; furthermore, the subjectivity from the border of radiopaque-radiolucent became the limitation of this research.

CONCLUSION

Our results showed that the defect diameter size in femurs treated with mangosteen peel extract at a dosage of 40 mg/kg was decreasing. This showed the healing process of fractures.

CONFLICTS OF INTEREST

The author reports no conflicts of interest.

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