A new device to produce a standardized experimental fracture in the rat tibia

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ABSTRACT

Objective: To develop and introduce a new device to produce a standardized closed experimental fracture in the rat tibia.

Methods: This study took place in the Research Laboratory, Medical Faculty, Dokuz Eylul University, in the year 2003. We include 20 healthy male white Wistar rats. After pinning both tibia of the rat intramedullary with the needle of a sterile injector without any incision, we tried to produce a fracture with the pendulum of the device, which was dropped in different angles in 9 rats. The tibial diaphysis of 14 rats in the main study were fractured at 60 degrees. After the fractures were confirmed radiologically, 4 tibia underwent pathological analysis to determine the degree of soft tissue damage and 24 tibia were examined in terms of histological fracture healing.

Results: Radiologically, this technique resulted in a transverse or short oblique bicortical fracture in the middle of the tibial diaphysis. The healing process was well adjusted with the classification of Allen. No noticeable soft tissue damage in the fracture region was demonstrated.

Conclusion: This method of producing an easy and reproducible fracture in a standard fashion without displacement and minimal soft tissue trauma in laboratory animals with this simple apparatus make it a useful technique for bone healing studies.

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Most of our present knowledge on fracture healing has been derived from the animal experiments.[1] In such studies, it is known that the fracture model should be standard and constructed in ethical ways. In an effort to produce an improved standard experimental fracture, in regard to fracture site, fracture line, degree of displacement, extent of soft tissue damage, rigidity of fixation[2] and time needed for union,[3,4] some investigators have spent their time to design standardized apparatus.[2,4] When we decided to study on fracture healing on laboratory animals, we required an apparatus to produce standardized fractures. So, we designed a simple and portable apparatus, which eliminated the need for both power accessories and incision to prenail the bone. The purpose of this report is to describe and introduce this apparatus. We also aimed to demonstrate the natural fracture healing process provided with this apparatus histologically.

Methods. The study met the criteria of the local ethics committee of our faculty and was performed in 2003 in the Research Laboratory of Dokuz Eylul University Medical Faculty in accordance with the ethics standards noted in "Principles of laboratory animal care".[5]

Fracture apparatus (Figure 1). The fracture apparatus consists of 2 major parts: a) an animal support table, and b) a pendulum with a chromium discus to produce the fracture. The dimensions of the animal support table are 28 x 50 cm. On this table in horizontal position, the leg of the animal is fixed with the help of the manacles made of plastic (**Figure 1** with label A) on both sides. These manacles are fastened with 4 butterfly screws, 2 just above the ankle, 2 at the thigh just above the knee (**Figure 1** with label B). The lower extremity is fixed in external rotation so as the pendulum can hit the tibia from the medial aspect. In this position, tibial diaphysis that will be broken should be at the middle of 2

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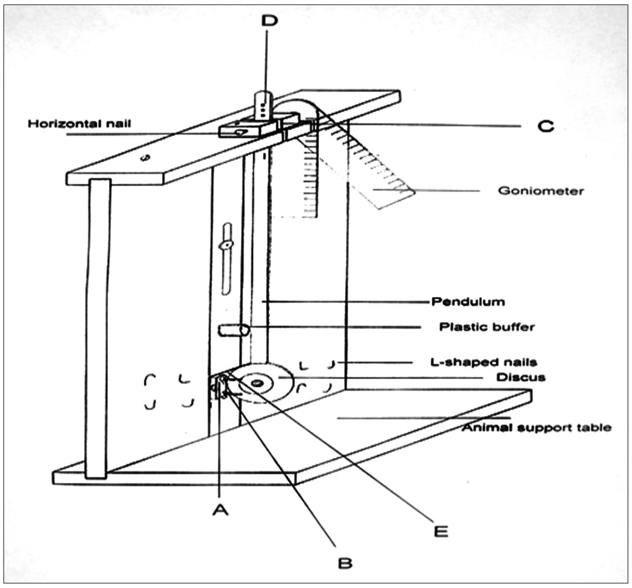

Figure 1 - Design of the fracture apparatus.

manacles. The body of the animal was fixed to the table with the help of ropes tied to L-shaped nails those were placed at both sides of the leg fixators. The pendulum has 2 parts: a) a discus made of chromium 382 g in weight and 65 mm in diameter connecting to, and b) a flat chromium rod 440 mm in length, 3 mm in thickness and 14 mm in width. The rod is held with a horizontal nail at the top of the animal support table in a cleft (**Figure 1** with label C), a little more than 3 mm in thickness, to allow the rod to move with ease. The point that the rod is hung can be changed upwards or downwards in a range of 40.5 - 44.5 cm with the help of the holes on it (**Figure 1** with label D). The middle part of the discus is thick and becoming thin at the edges. The edges are blunt and covered with a plastic material to reduce damage to the soft tissue. With the help of a goniometer replaced synchronously with the hanging point of the pendulum's rod, it is possible to measure the angle between the pendulum and animal support table, which determines the angle of the discus striking the bone. While a hand of the goniometer is fixed to the table in the cleft, the other hand is movable between 0-90 degrees angle. On the support table just at the back of the striking point of the discus, there is a part that can be changed in depth in an effort to prevent soft tissue damage (**Figure 1** with label E). A plastic buffer is placed on the table, 3 cm upwards to the striking point of the discus just across its rod to minimize the damage to the soft tissue and to prevent the discus from applying force after the production of the fracture.

Pilot study, anesthesia and fracture technique. In an effort to determine the dropping angle of the pendulum that breaks the bone in standard fashion in all rats, 9 healthy male white Wistar rats (outbredstrain of albinorats belonging to the species Rattus norvegicus) with a mean age of 5 months weighing 225g (185-253) in average were underwent breaking their both tibia until a standard, closed, transverse or short oblique fracture in the middle of the diaphysis was produced. Prepinning and fracturing was applied under general anesthesia produced by administration of pentothal sodium 30 mg/kg intraperitoneally. For prepinning, we used the stainless steel needle of a sterile injector with 0.8 mm in diameter and 38 mm in length. After scrubbing both knees with betadine solution, in 90 degree of flexion, we drived the needle in an antegrade fashion from the anterior part of the tibial plateau into the medullary cavity until resistance was encountered at the cortex above the ankle joint. Then the needle was cut flush with the skin. Then, the rats were placed on the table as mentioned above and with the table in perpendicular position, the discus was dropped respectively with an angle of 40, 50 and 60 degrees until the most convenient angle was supplied. The striking point was just at the medial part of the leg where the muscle tissue was thick. At 40 degree angle, we could not produce any fracture in 6 tibia of 3 rats and only 2 fractures in 6 tibia of 3 other rats were performed with 50 degrees angle. But, at 60 degrees, all 6 tibia of different 3 rats could be fractured in a standard fashion as proven with radiograms. So, we decided to fracture the bones with 60 degrees angle of the pendulum.

Main study. In the main study, 14 healthy male white Wistar rats including the 3 from the pilot study, with a mean age of 5 months, were used. The average weight of the rats was 219 g (164-270 g). After the prepinning procedure, the rat was placed on the table as mentioned above and the discus was dropped with an angle of 60 degrees (**Figure 2a**). Then, anteroposterior

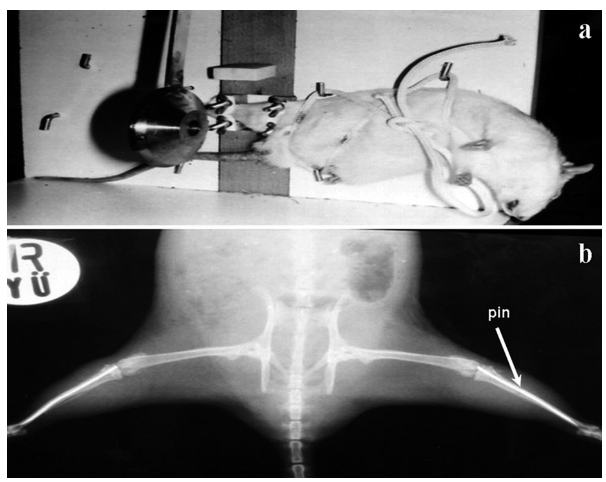

Figure 2 - Radiograms showing **a)** the fixation of the rat on the fracture table and striking point of the discus, **b)** both tibiae after fracturing procedure.

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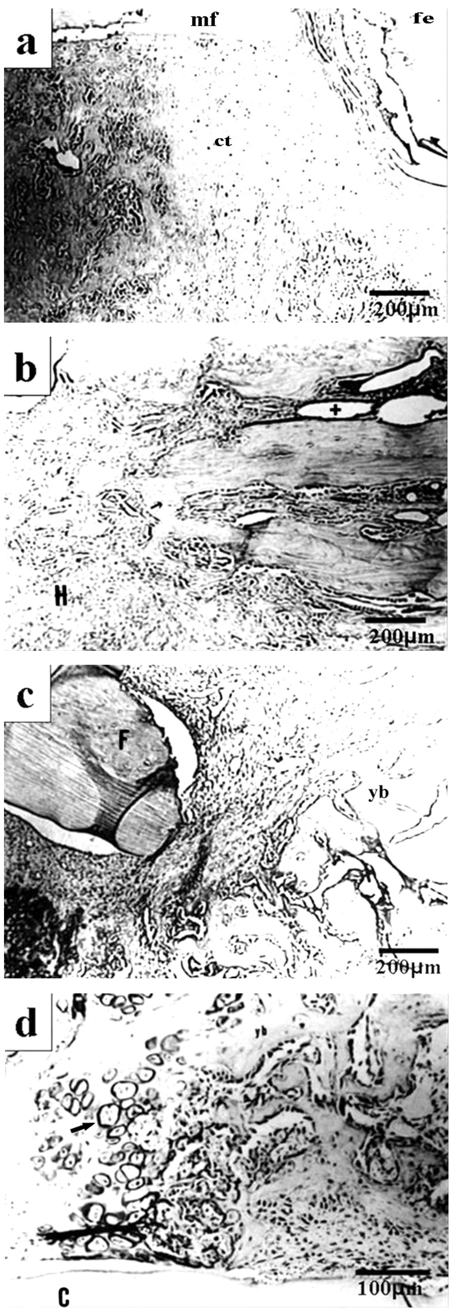

Figure 3 - Photographs **a)** 12th day, incomplete cartilaginous union. Grade 1 - Fragment end (fe), microfragment (mf), chondral tissue island (ct) (staining hematoxylin and eosin [H+E]). **b)** 2nd day, incomplete cartilaginous union. Grade 1 - intermedier and internal callus the area demonstrating hypercellularity (H), the tip of the ossifying tissue in the external callus (arrow), capillary proliferation in the healing area (+) (staining H+E). **c)** 21st day, complete cartilaginous union. Grade 2 - fragment (F), trabecular young bone tissue near the fragment tip (yb) (staining Erlich's H&E). **d)** 21st day, complete cartilaginous union. Grade 2 - cortex (C), trabecular young bone tissue (yb), degenerated cartilage cells substituted by the bone tissue (staining Erlich's H&E).

and lateral radiograms of the tibia were taken, in supine position with both hips abducted, to document the fracture pattern, to see if the pin is completely in the medullary cavity and to measure any angulation in the pin. The radiographic measurements were made with a standard goniometer. All animals were allowed free with full-weight bearing in their cages after awakening from anesthesia. We observed that the animals beared weight on both legs easily on the following day. We did not need to apply any antibiotherapy. Two hours after fracturing, 4 tibiae of 2 rats, with all covering tissue including skin underwent pathological analysis to determine the degree of soft tissue damage. The other 12 rats were randomly harvested 12, 21 and 28 days postoperatively. Both tibia were sent to the histology department after isolating from the surrounding soft tissues and fixed in 10% formalin. After 24 hours, the pins were removed without noticeable disturbance of the healing fracture sites and were measured again for any angulation. The samples were minimized by osteotomizing one cm proximally and distally from the fracture line. After fixating in formalin for 48 hours, the bones were decalcified in 10% nitric acid. Decalcification process was continued until appropriate hardness was obtained. After routine light microscopic paraffin blocks were obtained, 5-6 µm sections were evaluated under phase contrast microscope. Three of 5 preparates including 2 consecutive sections passing from the fracture line were stained with hematoxylin and eosin. In addition, the other 2 preparates were stained with Ehrlich's hematoxylin and eosin because of its specificity for the chondral islets. The histological evaluation of the fracture healing was made according to the system of Allen et al[5] independently by 2 histologists without knowledge of the sacrification day. Four samples reported differently by the histologists were evaluated again and a consensus was established on a single phase. The desired preparates were photographed by Olympus BH-2 photomicroscope.

Physical measurements of the apparatus. Moment of inertia of the homogenous metal rod with the mass m1 and length l in respect to the rotational axis at its tip is

$$I_1 = \frac{1}{2}m_1 l^2 + m_1\left(\frac{l}{2}\right)^2 = \frac{1}{3}m_1 l^2$$

and moment of inertia of the circular discus with the mass m^2, and radius R in respect to the same rotational axis is

$$I_2 = \frac{1}{2}m_2 R^2 + m_2(l + R)^2$$

Total moment of inertia of the system in respect to rotational axis is $I = I_1 + I_2$.

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When the compound pendulum, with the total mass of $m=m_1+m_2$ and radius of gyration of

$$k = \sqrt{\frac{I}{m}}$$

angle, is allowed free from a θ angle, its angular velocity is

$$\omega = \sqrt{\frac{2mgk(1-\cos\theta)}{I}}$$

and its linear velocity is

$$v = \omega l = \sqrt{\frac{2mgk(1-\cos\theta)}{I}}l$$

from the equality of

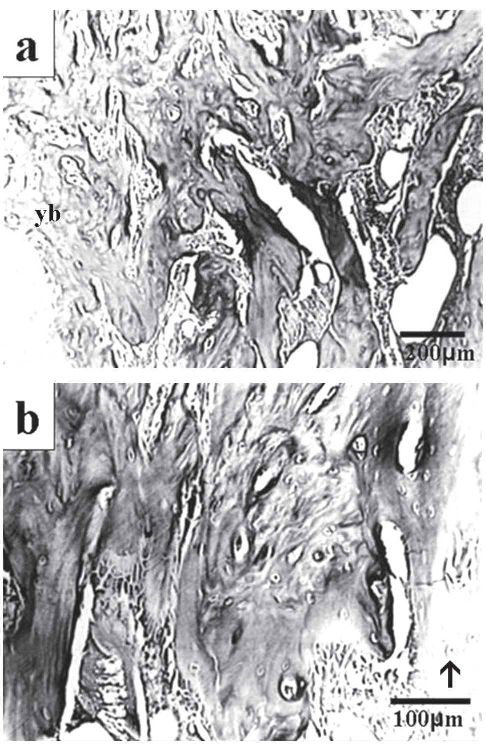
$$I_2 = \frac{1}{2}m_2R^2 + m_2(l+R)^2$$

at equilibrium position. According to the equalities above, m_1=144 g, m_2= 382 g, l = 44 cm, R=6.5 cm, g=9.8 m/s^2; $m = m_1 + m_2$= 526 g, I_1= 0.93 kg/m^2, I_2 = 0.09 kg/m^2, $I_1 + I_2$= 1.02 kg/m^2, k =1.63 m.

When θ = 60°, at the moment the discus passed from the equilibrium position, its angular velocity is ω=2.25 rad/s, and linear velocity is v=1.0 m/s.

Results. ***Clinical and radiological results.*** By radiographic evaluation, we observed that all pins were completely in the medullary cavity and a complete bicortical fracture was produced in the middle one-third of the tibial diaphysis in all rats. The fractures were in transverse fashion in 20 cases, and short oblique in 8 cases (**Figure 2b**). The average angulation of the pins was 5.57degrees as measured radiographically, and 5.28 degrees as measured after the pin was removed from the medullary cavity. Angulation of the nail of more than 10degrees occurred in 4 cases. All fractures were close and no superficial or deep infection could be diagnosed with clinical examination. No pins were migrated out of the skin. In 89.3% of the cases, there was no displacement in both radiograms. In 3 (10.7%) of the fractures, displacement was minimal, one in anteroposterior aspect, and 2 in lateral aspect. No rat was dropped from the follow-up because of any complication such as infection, migration of the pins or death.

Histopathological results. Healing grades of the fractured tibiae were listed in **Table 1**. At the end of the 12th day, 5 tibiae showed incomplete cartilaginous union while 3 had complete cartilaginous union. At the end of the 21st day, 5 tibiae demonstrated complete cartilaginous union while 3 had incomplete osseous union. At the end of the 28th day, 3 tibiae showed incomplete cartilaginous union whereas 5 demonstrated incomplete osseous union. No complete osseous union was observed until to the end of the experiment. Microphotographs of histological sections of the fractures in respect to healing grades can be seen on **Figures 3a - 3d**



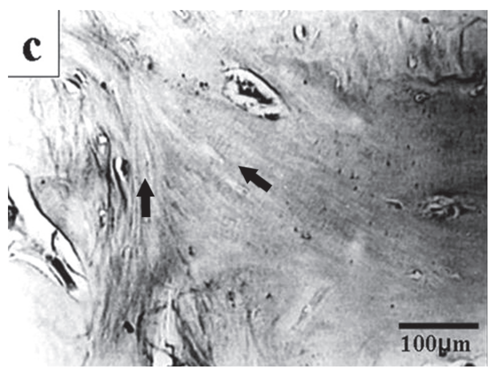


Figure 4 - Photographs **a)** 28th day, incomplete bony union. Grade 3 - subperiosteal trabecular young bone tissue (yb). Irregularity of the new bone tissue and abundance of the capillary sections are obvious (staining hematoxylin and eosin [H&E]). **b)** 28th day, incomplete bony union. Grade 3 - degenerated cartilage cell in the irregular trabecular young bone tissue (arrow) and abundance of the capillary sections, (staining Erlich's H&E). **c)** 28th day, incomplete bony union. Grade 3 - in this microphotograph, it is demonstrated that the collagen fiber bands in the bony callus are arranged in different directions (arrows), (staining Erlich's [H&E]).

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Table 1 - Healing grades of the fractured tibia according to 5-point scale of Allen et al.

Grades	12th Day (n=8)		21st day (n=8)		28th day (n=8)	
	R	L	R	L	R	L
Grade 0						
Grade 1	3	2				
Grade 2	1	2	2	3	1	2
Grade 3			2	1	3	2
Grade 4						

R - right tibia, L - left tibia

to **Figures 4a - 4c**. In consecutive sections of some bones, microfragments were observed in the fracture region (**Figure 3a**). After 2 hours, no pathological noticeable soft tissue damage and minimally focal extravasated blood in the fracture region were demonstrated.

Discussion. Early experimental fractures in laboratory animals were produced by manual loading, creating fractures that varied with respect to site, configuration and soft tissue damage because of the surprising amount of the uncontrolled force.[3,4] Ekeland et al[8-10] have introduced a special forceps to produce fractures by three-point bending6 and it was used in some experimental studies. Molster et al[11,12] have used open osteotomy with a circular saw in their experimental studies on fracture healing that was used by some other researchers.[13,14] Jackson et al[4] reported a method in which a rat femur was prepinned with an intramedullary wire and subsequently fractured with a blunt guillotine. This method resulted in a highly reproducible fracture site and configuration and was associated with minimal soft tissue trauma. The guillotine was driven by a pneumatic punch press powered by a cylinder of compressed air and forces were controlled by a pressure gauge and valve.[3] Greiff manufactured a fracture device to fracture the prenailed rat tibia.[15] This device was consisting of a guillotine with a blade made of stainless steel and they produced standard closed transverse or short oblique fractures with little displacement and angulation in the middle of the tibia. In 1984, Bonnarens and Einhorn designed a simple apparatus that eliminated the need for power accessories.[3] After prereaming of the femoral canal and prepinning in a retrograde fashion with 2 incisions on the knee and hip, they used their apparatus consisting of a guillotine ramming system. In 1993, Hiltunen et al[2] described their apparatus, a modification of Bonnarens and Einhorn's, to achieve a reproducible fracture pattern in the mouse tibia. In accordance with all these experimental studies described above, it is obvious that a closed fracture is superior to an open osteotomy through elimination of the added variable of local wound healing[3] such as increased local trauma and risk of infection.[4] When one has decided to build a model for closed fracture, they should have taken fracture site, fracture line, degree of displacement, extent of soft tissue damage, rigidity of fixation and time needed for union into consideration. To produce a standardized fracture, it is also important that same conditions have to be provided in the apparatus in each application. The present apparatus, created according to the same idea, has a different figure, with a discus bound to a flat metal rod that is allowed free from an angle and hits the tissue replaced on the striking point to produce a fracture. To make the discus always hit the same point at the same direction, the flat rod is replaced in a narrow cleft to limit its movement to the right and left. To avoid from repetitive forces on the broken bone, a plastic buffer was placed on the table, just 3 cm upwards to the striking point of the discus to minimize the probable damage to the soft tissue. Also, the blunt design of the discus' edges and covering it with a plastic material resulted in a minimal soft tissue trauma. As approved by the pathological examination, there was no noticeable soft tissue damage in the fracture region just like described previously.[3] It is obvious that the condition of the surrounding soft tissue is very important for fracture healing. The present fracture apparatus is mostly suitable for tibia, but it can be modified for the other long bones. As described in the results, the method consistently produced closed fractures with no or minimal displacement mostly in transverse or short oblique fashion in the midshaft of the rat tibia. In the present study, fracture healing process was well-adjusted with the classification of Allen et al. However, some differences have been observed between both tibiae of the same rat in regard to healing. In our opinion, some microfragments, which are unavoidable can be accused for this. In some consecutive histologic sections, these particles were demonstrated. If these fragments are as large as that they cannot be resorbed, they may remain in callus formation and affect the healing negatively by making it remain behind the normal grading that it must stay. The fixation technique used in the present study is analogous to a common clinical method used in treatment of tibia and the other long bones. Experimental and clinical studies have shown that fracture repair would not be prevented by this method.[2,4,16] The ability of the rats to bear weight immediately after anesthesia and the pathologic findings of no soft tissue damage support the conclusion that this technique produces a fracture of tibia without significant associated injuries.

In summary, this method of producing an easy, fast and reproducible fracture in a standard fashion without

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or with very slight displacement and minimal soft tissue trauma in laboratory animals with this simple and cheap apparatus make it a useful technique in laboratory animals for bone healing studies. If the dimensions of the apparatus are changed, it can also be used to produce standard fractures in mice and rabbits.

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