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Behavioral Assessment of Post-Operative Pain after Femoral Condyle Surgery in Male Laboratory Rabbits

Sesha Subramanian Murugan[1*], Suprith Surya[2], Sukumaran Anil[3] and Jayachandran Venkatesan[1]

*[*1]Biomaterials Research Laboratory, Yenepoya Research Centre, Yenepoya Deemed to be University, Deralakatte, Mangaluru, 575018, India*

[2]Advancement Surgical Skill Enhancement Division, Yenepoya Deemed to be University, Deralakatte, Mangaluru, 575018, India

[3]Department of Dentistry, Oral Health Institute, Hamad Medical Corporation, College of Dental Medicine, Qatar University, Doha, Qatar.

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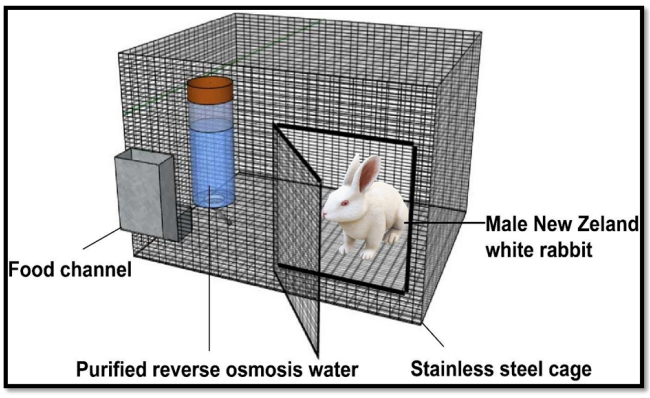
ABSTRACT

Monitoring the behavior patterns of the animals in the testing procedure provides significant knowledge for the researchers to conduct the animal experiments. This study aimed to compare the behavior activities that could be identified in preoperative and post-operative investigations during the implantation of bone graft material into the femoral condyle of the hind limbs. The behavioral data were obtained from three male New Zealand white rabbits, and the average mean and standard deviation were calculated. The implantation procedure was carried out under proper anesthesia and analgesic protocols. Implants were placed in the femoral condyle of both the right and left hind limbs. The behavioral activities were compared with preoperative and post-operative procedures to determine changes in behavioral activity caused by pain. The study results suggest that the normal behavioral activity of eating food, drinking water, walking, digging, leaping, grooming, writhing, licking, staggering, and relaxing were changed after surgery. In addition, significant changes are observed in the post-operative produce, which can be overcome by following the management and treatment procedure.

Keywords: Behavior, Bone grafting material, Pain, Rabbit, Surgery

GRAPHICAL ABSTRACT



INTRODUCTION

In vivo research with an animal is essential to translate the materials or drugs to commercial application. Animals should be economical, readily available, adapt to environmental conditions, easy to handle and easy to manage. In addition to this, the animals must be study with robust immune system. The animal model has proven to be an effective alternative for humans in determining the biological role in tissue integrity and regeneration [1].

Due to the texture and composition of the bone, the rabbits are the most widely used laboratory animal in pre-clinical research investigations to evaluate bone-implant interaction and bone- related research, including osteoporosis, pre-clinical bone implant material testing, and bone trauma studies [2-4]. We tested bone implant material in a male New Zealand white rabbit for its efficacy in regenerating bone. The animal's behavior may alter before and after surgery when working with the animal model. Hence, the behavior changes of rabbits during preoperative and postoperative procedures are presented in the current study.

MATERIALS AND METHODS

Corresponding author: Sesha Subramanian Murugan, Biomaterials Research Laboratory, Yenepoya Research Centre, Yenepoya Deemed to be University, Deralakatte, Mangaluru, 575018, India, Tel: +91-8012080219, +91-965992165; E-mail: seshae5@gmail.com

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Three healthy rabbits weighing between 1.8 kg and 1.9 kg were procured from Adita Biosys private limited (Tumakuru, Karnataka, India). The rabbits were kept in the animal house at the department of pharmacology at Yenepoya (Deemed to be a university). Individual standard laboratory rabbit cages (V and C scientific traders, 610 x 460 x 450 mm; length x width x height) were used for the rabbits, kept in a temperature-controlled room (24°C) with 50% humidity. The bedding materials were not given since the cage's 15 mm diameter apertures have a significant risk of allowing the materials to fall into the tray below. The Institutional animal ethical committee approved the use of New Zealand male rabbits in the study (YU/IAEC/12/2021).

The animal's behavior was observed and recorded using a CCTV security camera during the quarantine and post-surgery periods. Furthermore, the behavior of the animals was monitored during the animal's care, handling, weighing, and cleaning. Each rabbit was weighed every seven days. The cages were cleaned and disinfected every day using a disinfectant solution.

EXPERIMENT DESIGN

The animals were weighed separately and recorded on the day of the surgery **(Table 1)**. Then, the individual animal was moved from the holding room to the procedure room. Intramuscular injections of 35 mg/kg ketamine and 5 mg/kg xylazine were administer to anesthetize the animal. The surgery was carried out on both the left and right hind limbs, with the left femoral condyle being done first and the incision being closed, followed by the right femoral condyle region with the same procedures [5]. Throughout the procedure animals were maintained under inhalant anesthetics, 2.5 % isoflurane. The surgery involved operating two hind limbs and lasted about three hours in a sterile environment.

Table 1. Illustrates the weight (kg) of a preoperative, postoperative and at the time of surgery.

S No	The preoperative phase of surgery (kg)	During operation (kg)	The post-operative phase of surgery (kg)
1	2.25	2.35	1.45
2	2.35	2.40	1.85
3	2.15	2.20	1.70

SURGICAL PROCEDURE

After immobilizing the animals in the procedure room, the hair follicles on the femur area of the hind limbs were shaved using a razor blade proper aseptic condition was maintained. The incision was made on the paramedial aspect of the patellar region (knee joint) with blade No. 22. Later, the fascia was incised, ligaments and patella were reflected and the femoral condyle area was approached. A periosteal elevator was used to locate the periosteum. The pilot drill was initially made using drill bits, and the implant site was subsequently extended to 5 mm diameter using low-speed rotating drilling and saline irrigation. After that, the implant was placed on the defect site, and the fascia was closed with a 3.0 vicryl suture. Next, the skin was stitched using 3.0 nylon sutures, and the surgical site was cleaned. Finally, a meloxicam injection was administered for pain management. After surgery, the rabbits were kept in the operating room for at least 30 min. Following the surgical procedures, the animals were returned to their cages and intensively monitored for specific signs of distress.

BEHAVIOR STUDY ANALYSIS

The following behaviors were examined in the behavioral data recordings: eating food, drinking water, walking, digging, leaping, grooming, writhing, licking, staggering, and relaxing [6,7]. Each animal's behavior was monitored for 14 d (in min) before and after surgery.

Eating food

Rabbits have a habit of eating in a hunched position, and it is an essential habit of enjoying food. Therefore, the animals were fed protein pellets, fresh vegetables with carrots, cabbage, cauliflower, and baby sorghum leaves on an alternate day at eight hours intervals. The uneaten food was routinely removed.

Drinking water

The purified reverse osmosis water was filled and kept in water bottles and a small pot for animals during the quarantine period. We observed that rabbits drank water from water bottles, and animals preferred to drink water from a small pot. The drinking habit may aid the rabbit's digestion, absorption of nutrients, and waste elimination by urine excretion. It also aids in keeping the rabbit's body temperature within a normal range (37.5°C to 40°C).

Walking

Walking is the rabbit's most essential habit. Stepping inside the cage provided the rabbits with comfort, enjoyment, and a way to burn off energy. As a result, the rabbit moves towards the front, corners, and backside of the cage.

Digging

Digging is a kind of exercise and a necessary physical and psychological habit in rabbits. For example, rabbits who have been confined to a cage for days on end are more likely to dig to release stress.

Leaping

Leaping is a natural behavior of rabbits when they feel comfortable with their environment. The hind limbs of rabbits are quite strong, allowing them to jump. They can

propel themselves forward with a single push from their back legs. They usually land on their front legs, which aids balance, while their rear legs leap forward into position to push off for the next jump.

Grooming

Grooming is the primary habit, which generally includes licking the paws, and washing the nose, ears, body, face, legs, tail, and reproductive organs. Typically, rabbits were groomed more regularly to remove parasites and clean unreachable areas of the face or back of the neck [8]. When grooming the ears, face, and back body, the animal stands typically and bears weight on the hind limbs.

Writhing

Writing is a stretching behavior in which an animal's muscular body tenses to one side, the hind legs elongate, and the belly compresses towards the cage. This type of behavior isuncommon, but it is easily noticeable [9-11].

Licking

Rabbits lick themselves extensively to groom themselves. Every day, rabbits spend hours grooming themselves. Excessive licking may indicate that the animal is ill. The licking has been observed mainly on the paws, belly, and ankle.

Staggering

Rabbit stumbling or staggering may be followed by head tilt (or "wry neck"), in which the rabbit tilts its head to one side. It indicates that the animal is in distress.

Relaxation

The rabbits relaxing behavior was seen in a variety of postures. While sleeping and relaxing, the animals used to relax and extend their entire body, with the forelegs shuffled half stretch and the hind legs extending backward. At this point, the animals' ears are twisted to the back, and their eyes are partially closed in some instances. The animals' entire bodies are occasionally relaxed on one side, with their fore and rear legs extended out in front, keeping them comfortable and enabling them to rest their heads on the cage floor for a few minutes. To make themselves more comfortable in a sloping position, the animals would sometimes relax their anterior bodies on the lateral side and stretch out the hind and forelimbs straight, which made them comfortable in a sloping position. Most of the time, rabbits tuck their hinds and forelimbs within their bodies and lie down with their front paws facing forward and their back legs sticking out sideways, or they fully extend their bodies and stretch out their back legs, pointing their front paws forward. Often the rabbits crouched their bodies with their hind legs curled under and their forelegs extended or squatted on their forelegs with their hind legs extended. Sometimes the rabbits flatten their abdomens into the cage and bend their forelegs to sit comfortably while the foot area is straight and the rear legs are tucked in. The rabbits were used to sleeping more lightly and for a shorter time when resting. While sleeping, their legs were curled under their bodies, and their eyes were half / partially closed most of the time.

RESULTS AND DISCUSSION

Eating food

The rabbits eat more green leaves than the other feeds of protein pellet, carrot, cabbage or cauliflower, and baby sorghum in a hunching position during our observation. The animals hold the leaves in their forelimbs and bite them for a prolonged period. Their ears are upright when eating in a hunching position, and they groom their lips, vibrissae, nose, chest, thorax, forelimbs, legs, and toes with their forelimbs. During the 7 d preoperative periods, the rabbits kept eating for 2912 min. However, the time spent eating was decreased to 1008 min after surgery **(Figure 1)**. As a result, one week after surgery, the animal's weight was dropped to 1.6 kg with an average 0.65 kg reduction in their respective body weights.

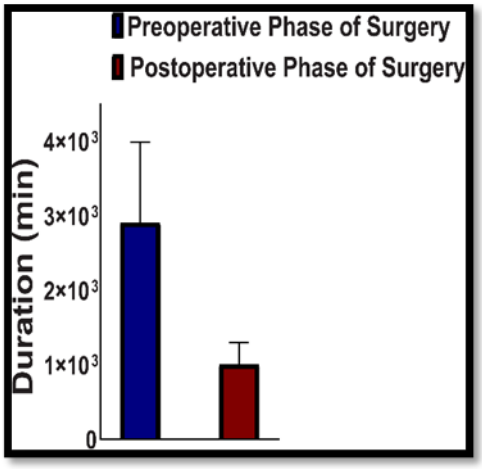

Figure 1. The behavior of eating food, preoperative and postoperative phase of surgery.

Drinking water

The rabbits drank more water during the preoperative period, with 672 min consumed in one week, but this was drastically reduced during post-operative surgery for two days. Dehydration was evident in dark yellow urine, which could be due to the anesthetic administered during the surgery. Later, the animals started drinking water and set 392 min during the one-week post-operative surgery **(Figure 2)**. The urine excretion was normal being found to be a pale-yellow color. Drinking water from a pot is faster than drinking water from a bottle during the experiments. Furthermore, sometimes the rabbits never drink water when eating the greeneries, owing to moisture in the leaves. Grooming was also seen after drinking and eating at different times, demonstrating the rabbit's cleaning personality.

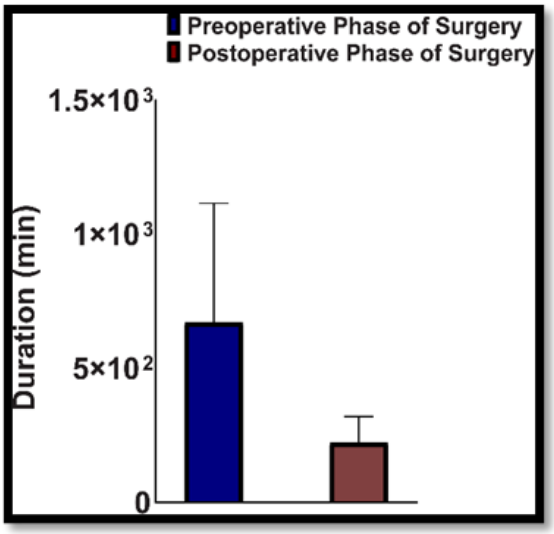

Figure 2. The behavior of drinking water, preoperative and postoperative phase of surgery.

Walking

The walking time during the preoperative period was 1344 min, and it was decreased to 168 min following surgery **(Figure 3)**. The activity demonstrates that the animal does not feel comfortable walking due to aberrant changes in the body, including the discomfort.

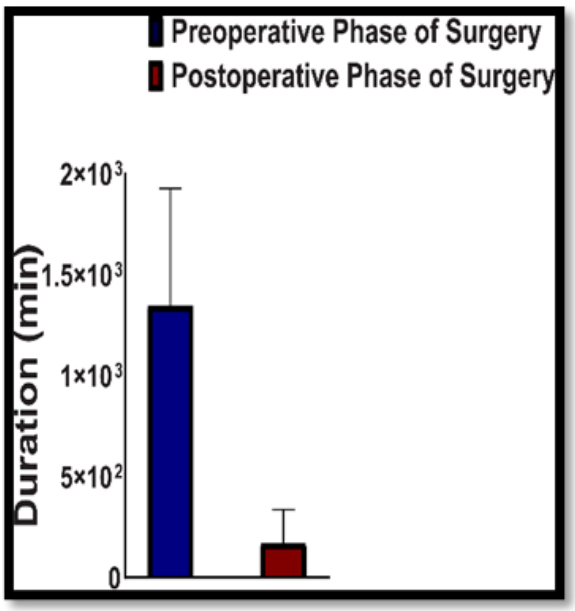

Figure 3. The behavior of walking, preoperative and postoperative phase of surgery.

Digging

The rabbits were healthy and dug an average of 16688 times during the preoperative surgery period, but this number dropped to zero during the postoperative period **(Figure 4)**.

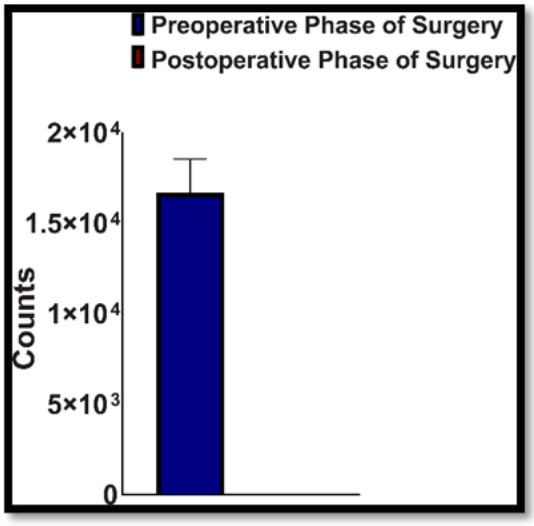

Figure 4. The behavior of digging, preoperative and postoperative phases of surgery.

Leaping

Animal jumps 2408 times before surgery, but it was decreased to 112 times after postoperative **(Figure 5)**. The reduction of jumping in rabbits due to the operation on the rabbit's femur condyle area.

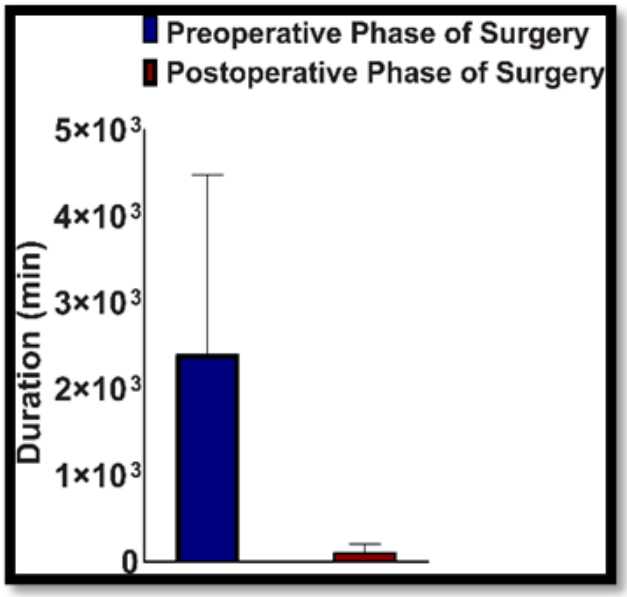

Figure 5. The behavior of leaping, preoperative and postoperative phase of surgery.

Grooming

Grooming activities were recorded for 3136 min before surgery, indicating that the animals were active and self-caring on hygiene. However, following surgery, the grooming activities were changed and reduced to 448 min **(Figure 6)**, possibly due to acute discomfort in the hind limbs.

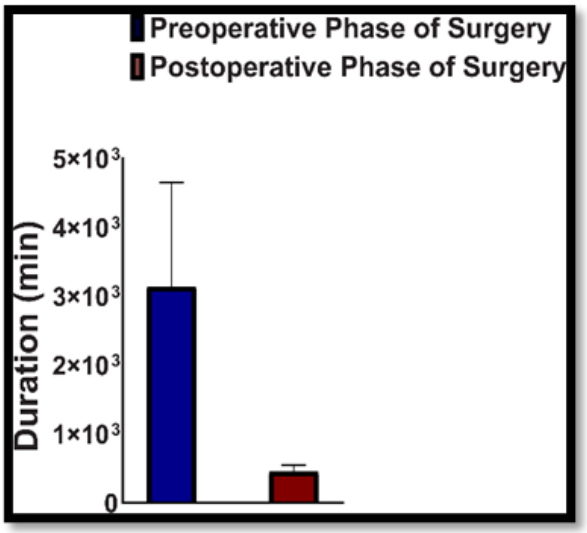

Figure 6. The behavior of grooming, preoperative and postoperative phase of surgery.

Writhing

The writhing and twitching behaviors were non-existent during the post-operative period. The animal writhed 560 times during preoperative, but this was reduced to 168 times due to surgery **(Figure 7)** .

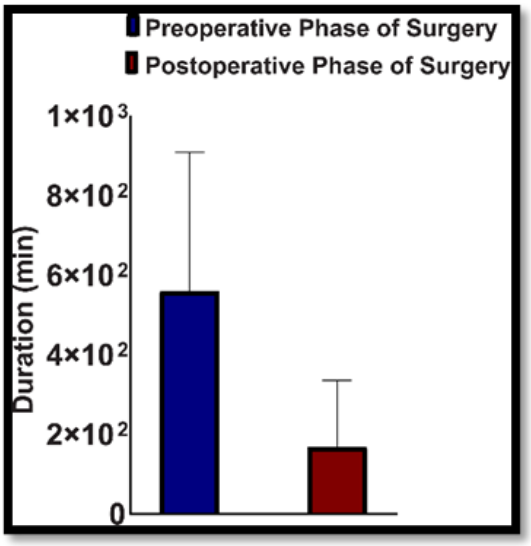

Figure 7. The behavior of writhing, preoperative and postoperative phase of surgery.

Licking

The rabbits' licking behavior was observed to clean themselves. The licking on the surgical site after the post-operative period might be related to pain. In addition, the rabbit licks a specific area or grooms itself to moisten its fur, increasing the chances of microbial infection at the surgical site. Licking before surgery witnessed 2016 min, whereas licking after surgery accounted for 560 min **(Figure 8)**.

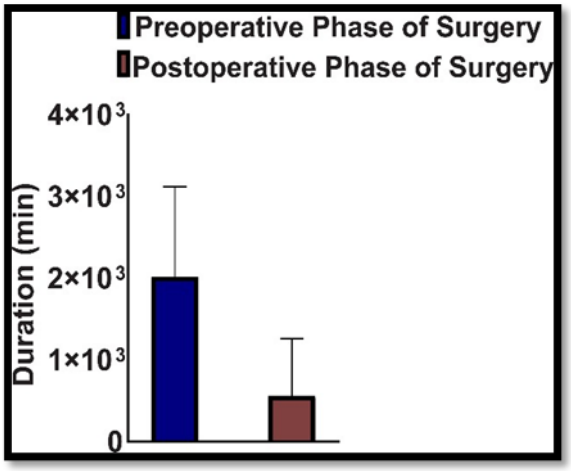

Figure 8. The behavior of licking, preoperative and postoperative phase of surgery.

Staggering

The animal loses strength and staggers more frequently after surgery, observed up to 784 times. As a result of the pain, their existing drooping appearance may become much more evident. There is no evidence of the staggering behavior before surgery **(Figure 9)**.

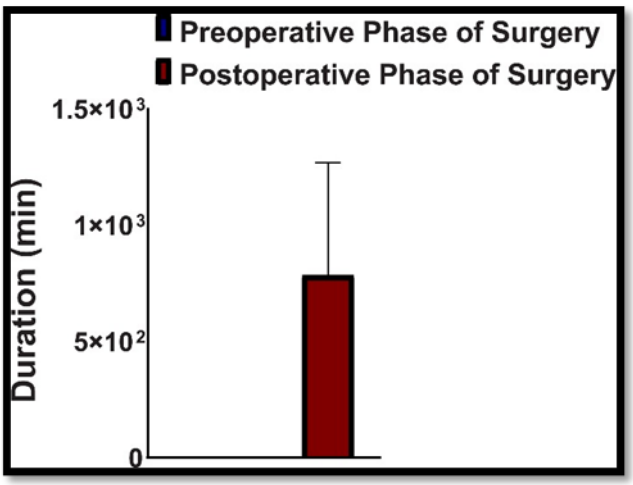

Figure 9. The behavior of staggering, preoperative and postoperative phase of surgery.

Relaxation

It was evidenced the relaxing behaviors of the rabbits were disturbed after the post-surgery. The animals were restless and aggressive over the first two days. Administration of anesthetics and the surgery could have exhausted the animal. The preoperative surgery was recorded as 1232 min during the entire body relaxation, but because of the surgery in the condyle region of the hind limbs the body relaxation time in ventral position by stretching the limbs were completely reduced **(Figure 10 (a))**. Pre-surgery relaxation in a sitting position accounted for 2296 min, and it was decreased to 925 min during the post-surgery period **(Figure 10 (b))**. Most of

the time, the animals tuck their legs beneath their bodies, documented more than 8064 times during the post-operative period than the preoperative period of 1456 min **(Figure 10 (c))**. This might be linked to discomfortness in that position. Instead, the rabbits prefer to relax by facing their abdomens towards the cage, with minimal difference between the postoperative and preoperative periods **(Figure 10 (d))**. The rabbits sleeping habits were disrupted after surgery that decreased from 3864 min to 1288 min, which might be due to pain.

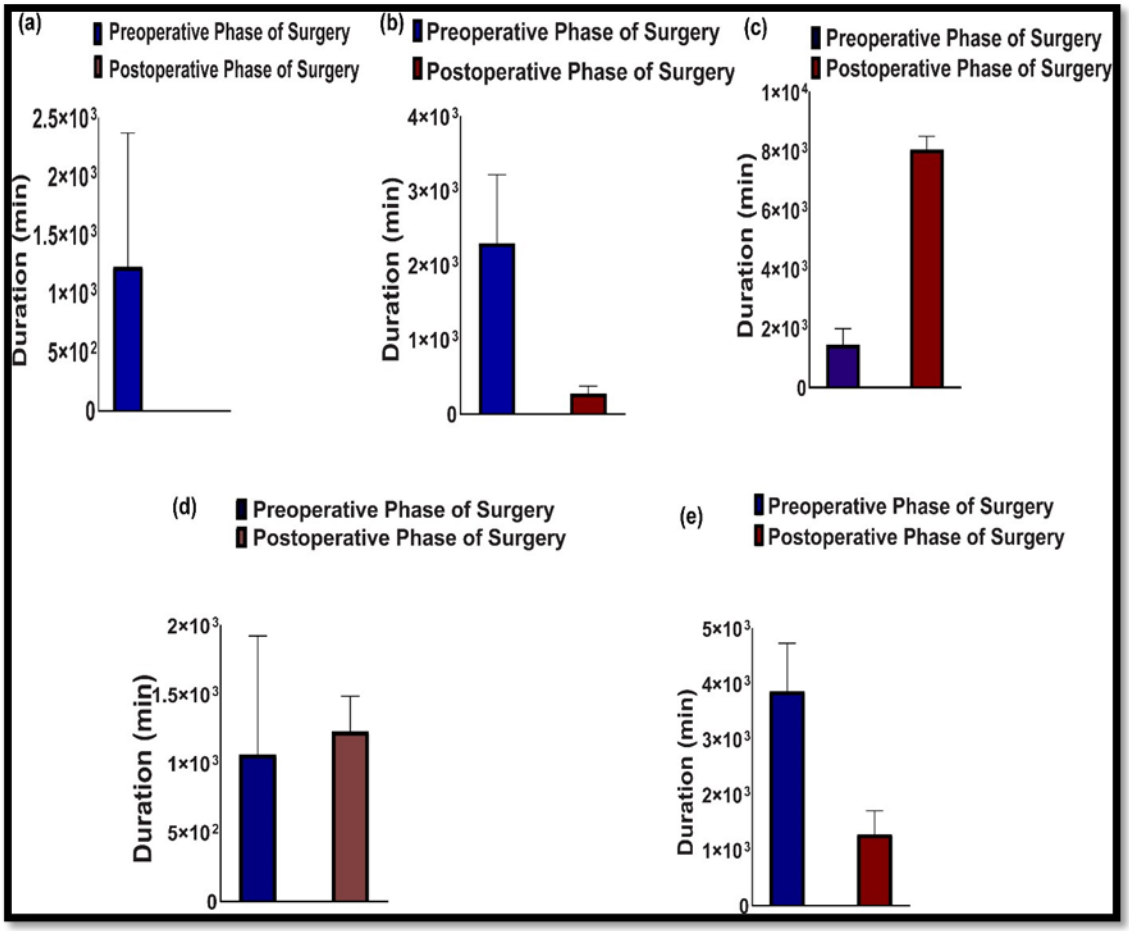

Figure 10. The behavior of relaxation (a) total body relaxing (b) sitting relaxing (c) legs tucked under the body, (d) relaxing flat on the cage, and (e) sleeping.

CONCLUSION

Rabbit behavior after the bone implants were observed in the current study. The animal's health, urine excretion, weight, food consumption, sleep, and feces were observed during preoperative and postoperative. Following surgery, the fecal and urine excretion was declared normal other behaviors have altered. The bunnies were quite abnormal after the procedure on the condyle region. The lack of activity might be owing to the surgical pain and anesthesia in the body, that produces discomfort. Lack of enrichment might be the reinforcing factor for the altered behavior. Animals had minimal food and irregular or absence of fecal excretion after the operation for 48 h. A betadine antiseptic solution was applied to the surgical site to prevent infection and aid wound healing. We noticed that the wound had begun to heal after 7d. Rhinitis was noticed in the rabbits during the course of experimentation, which was treated accordingly. Post-operative care must be taken with considerable caution, including keeping the animals in dry area/cages, proper cage cleaning, and changing the water and food twice a day to prevent infection.

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