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## STUDY OF THE EFFECTIVENESS OF THE EXPERIMENTAL MODEL OF IMMOBILIZATION CONTRACTURES IN RATS

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### Abstract

Immobilization contractures are a common complication that can arise following prolonged immobilization of a joint or limb. They occur due to a combination of factors, including muscle atrophy, joint stiffness, and tissue adhesions. The aim of the study was to study the effectiveness of the experimental model of immobilization contractures in rats. The experimental study was conducted on 21 non-linear white male rats aged 6 months. We used 4-week plaster immobilization to evaluate the severity of changes in the knee joints of experimental rats. The immobilization and post-immobilization period in our study lasted 4 weeks each. Every week, the angles of extension and flexion, range of motion and severity of contracture were measured. During the period of immobilization, there was a progressive impairment of the function of the immobilized knee joints of all experimental animals. Thus, at the end of the 4<sup>th</sup> week of immobilization, the limits of flexion and extension on the immobilized limb gained high statistical significance in comparison with both the opposite intact limb and with 1st week of immobilization. In the post-immobilization period (from the 5<sup>th</sup> to the 8<sup>th</sup> week of the study), a progressive recovery of the studied indicators was observed. The indicators of extension and flexion of the limbs after immobilization were significantly differed from the initial values, but were also significantly better than the values at the end of the immobilization period. As for the range of motion, this indicator for both limbs was significantly higher than the values at 4<sup>th</sup> week of the study, but was also significantly lower than the initial values. Limitation of movements after immobilization was statistically significant both when compared with the opposite limb and when compared with baseline and 4<sup>th</sup> week of immobilization data. Experimental model of 4-weeks plaster immobilization of the knee joint allows to create a persistent pronounced limitation of movements and can be used for further study of various methods of prevention and treatment of immobilization contractures.

**Keywords:** *knee joint; immobilization contractures; experiment; rats.*

### INTRODUCTION

Immobilization contractures are a common complication that can arise following prolonged immobilization of a joint or limb [1]. They can arise following a range of injuries, including fractures, dislocations, and soft tissue injuries. Immobilization contractures can occur when a joint or limb is immobilized for an extended period, such as in a cast or brace, or as a result of prolonged bed rest. They occur due to a combination of factors, including muscle atrophy, joint stiffness, and tissue adhesions [2].

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It is essential to understand the relevance of this problem, both for patients and for the broader healthcare system. Immobilization contractures can be a significant problem for patients, as they can result in pain, limited range of motion, and reduced function [3; 4]. The severity of this condition can vary, with some patients experiencing only mild stiffness, while others may have severe, debilitating contractures that require surgical intervention [2].

Also it can have a substantial impact on quality of life of patients [5]. They can result in chronic pain, reduced mobility, and a decreased ability to perform daily activities. Immobilization contractures can also have a significant psychological impact, as patients may feel frustrated, anxious, or depressed due to their reduced function and mobility [6].

From a healthcare system perspective, immobilization contractures can be costly to manage.

Such patients may require multiple interventions, including physical therapy, occupational therapy, and sometimes surgical procedures, all of which can be expensive. Moreover, it can result in prolonged hospital stays, which can further increase healthcare costs [7].

Preventing immobilization contractures is crucial to minimizing their impact on patients and the healthcare system. Early mobilization, physical therapy, and range-of-motion exercises can all help prevent the development of this complication [1; 8].

The need to solve this problem dictates the search for an adequate animal model of immobilization contractures, which would be closest in terms of conditions and results to that observed in patients.

One of such models, in our opinion, could be plaster immobilization.

The aim of the study was to study the effectiveness of the experimental model of immobilization contractures in rats.

**Materials and Methods**

All experiments were performed in accordance with the “Regulations on the use of animals in biomedical experiments” with the permission of the Bioethics Committee and in accordance with Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes”. The study protocol was approved by the Committee on Bioethics, National Pirogov Memorial Medical University, Vinnytsya, Ukraine.

The experimental study was conducted on 21 non-linear white male rats aged 6 months. Before immobilization, the angle of extension and flexion of the knee joint was measured for all animals. Im-

mobilization of the hind limb at an angle of 140° was performed by applying a plaster bandage under anesthesia.

To determine the amount of movement in animals, the immobilization bandage was removed once a week, the skin condition was examined and, if necessary, treated with an antiseptic. Markers were placed on the hip, knee and hock joints. To determine the bending angle, the hind limb was maximally bent. To determine the extension, a weight of 50 grams was tied to the hock joint.

The animals were photographed and the angle between the markers on the pictures was measured. A similar procedure was performed for the opposite limb. After the measurements were completed, the immobilization bandage was re-

applied. Measurements were taken at 1-week intervals during the 4 weeks of immobilization and 4 weeks after the end of immobilization.

The range of motion was determined based on these measurements of the extension and flexion angles. Joint contracture was calculated as the difference between the determined range of motion and the range of motion before the start of the experiment for each animal individually.

The obtained data of instrumental studies were processed statistically using the IBM SPSS Statistics 20.0 application program package (USA). The mean (M) and standard deviation (SD) were calculated. Comparison of parameters of contralateral limbs was performed using the T-test method for repeated measurements.

**Results and Discussion**

The values of the angles of bending and extension, as well as the volume of movements in the knee joints of experimental animals are graphically shown in *Figures 1–3*.

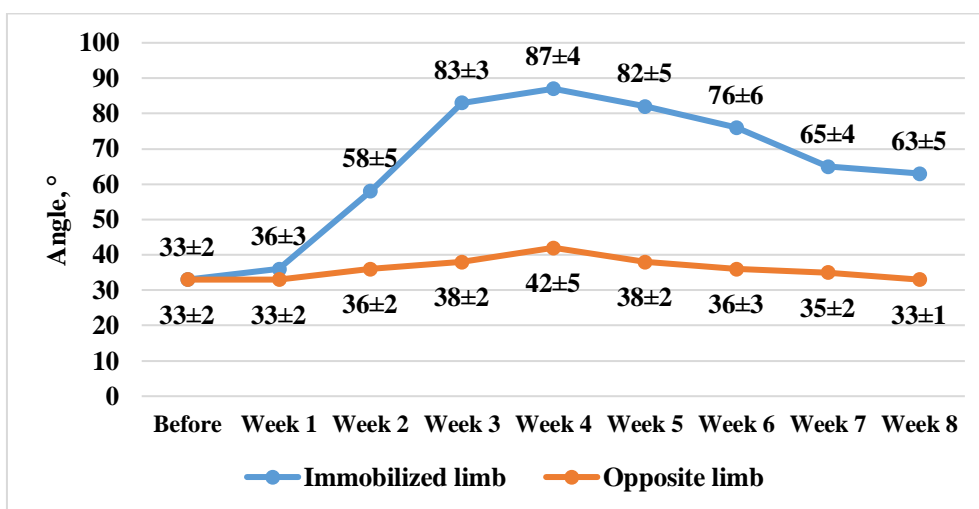


Fig. 1. The value of the angles of maximum extension in the knee joint of experimental animals.

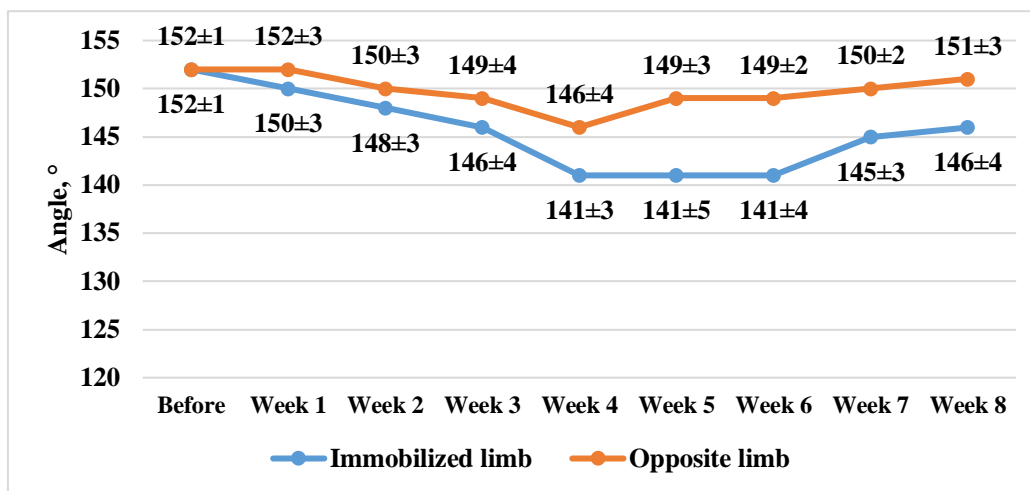


Fig. 2. The value of the angles of maximum flexion in the knee joint of experimental animals.

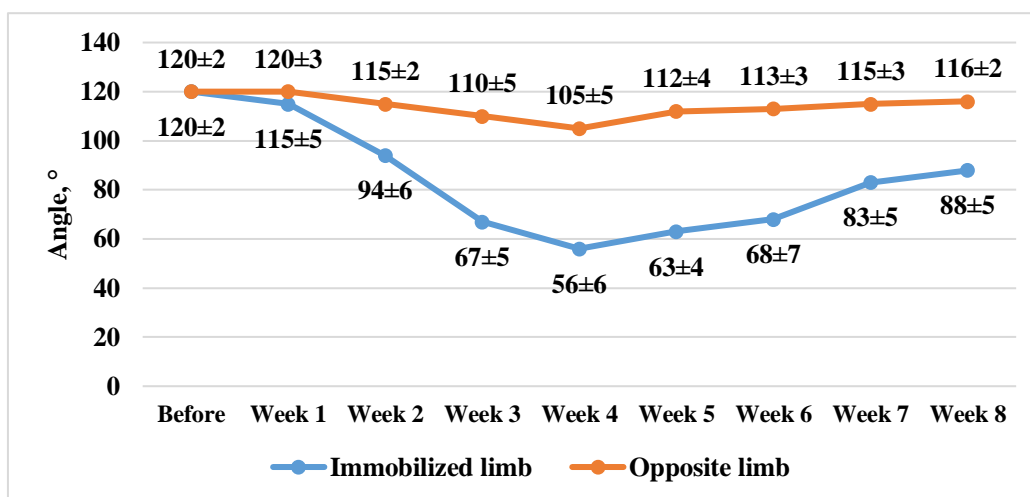


Fig. 3. The volume of movements in the knee joint of experimental animals.

Before the start of the experiment, the range of motion in the knee joint was measured in rats. The results of the statistical analysis showed that all the studied indicators were the same for both knee joints in each of the experimental rats.

At the end of 1<sup>st</sup> week, flexion ( $p=0.024$ ) and extension ( $p=0.010$ ) limitations of the immobilized limb reached a statistically significant level compared to the contralateral intact limb. The total range of motion of the immobilized limb ranged from  $106^{\circ}$  to  $120^{\circ}$  and was statistically significantly ( $p=0.002$ ) different from the values for the intact limb. Contracture during 1st week of the experiment averaged  $5^{\circ}$  (from  $0^{\circ}$  to  $13^{\circ}$ ). Moreover, a slight restriction of the range of motion was observed in both knee joints. In comparison with the control values before the beginning of the experiment, there were statistically significant changes in the angle of extension ( $p=0.041$ ), the angle

of flexion ( $p=0.040$ ) and the total range of motion ( $p=0.011$ ) of the immobilized joint. At the same time, no significant changes were observed in the opposite knee joint ( $p>0.05$ ).

In general, during the period of immobilization, there was a progressive impairment of the function of the immobilized knee joints of all experimental animals. Thus, at the end of the 4th week of immobilization, the limits of flexion ( $p<0.001$ ) and extension ( $p<0.001$ ) on the immobilized limb gained even greater statistical significance in comparison with both the opposite intact limb and with 1st week of immobilization. A similar tendency was demonstrated by indicators of the volume of movements in the joint. The total range of motion of the immobilized limb ranged from  $41^{\circ}$  to  $67^{\circ}$ , for the intact limb – from  $93^{\circ}$  to  $111^{\circ}$ . At the same time, the differences were statistically significant ( $p<0.001$ ). Also, statistically

significantly ( $p < 0.001$ ), these indicators differed from the indicators before the beginning of the study. Contracture on the 4th week of the experiment was  $(63 \pm 7)^\circ$  for the immobilized limb and  $(17 \pm 6)^\circ$  for the intact limb. Movement limitation was statistically significant ( $p < 0.001$ ) both when compared between opposite limbs and when compared with baseline data.

In the post-immobilization period (from the 5th to the 8th week of the study), a progressive recovery of the studied indicators was observed both for the immobilized limb and for the intact limb.

Thus, the indicators of extension and flexion of the limbs after immobilization, although significantly ( $p < 0.001$ ) differed from the initial values, but were also significantly ( $p < 0.001$ ) better than the values at the end of the immobilization period. Indicators of extension and flexion of intact limbs were not significantly different from the initial values ( $p > 0.05$ ). As for the range of motion, this indicator for both limbs was significantly ( $p < 0.001$ ) higher than the values at 4<sup>th</sup> week of the study, but was also significantly lower ( $p < 0.001$  and  $p = 0.015$  for immobilized and intact limbs, respectively) than the initial values. Overall range of motion ranged from  $79^\circ$  to  $92^\circ$  for immobilized limbs and from  $112^\circ$  to  $120^\circ$  for intact limbs. The contracture at the 8th week of the experiment was  $(33 \pm 5)^\circ$  for the immobilized limb and  $(4 \pm 3)^\circ$  for the intact limb. Limitation of movements after immobilization was statistically significant ( $p < 0.001$ ) both when compared with the opposite limb and when compared with baseline and 4<sup>th</sup> week data. Limitation of movements of intact limbs was significantly ( $p < 0.001$ ) lower than the indicators at the 4<sup>th</sup> week of the study and was not significantly different ( $p > 0.05$ ) from the initial data.

The data obtained during the study coincide with the results of other researchers. Thus, in the work of Chimoto et al. [9] immobilization of the knee joints in rats using an internal fixator in a flexion position of  $150^\circ$  was performed. The aim of the study was to develop an animal model of joint contracture to further investigate the progression of arthrogenic limitation of motion after immobi-

lization. The study demonstrated a rapid progression of joint contracture up to 8 weeks. Although our experiment lasted only 4 weeks, we observed the progression of the contracture every week. Similar trends were demonstrated by other authors, in particular Kojima et al. [10].

Regarding the restoration of range of motion after immobilization, according to Trudel et al. [11] complete recovery of joint movements after 30 days of immobilization does not occur even on the 8th week of the post-immobilization period. At the same time, Sato et al. [12] reported a 32-week period required for full movement recovery after joint fixation in rats.

In general, the experimental model of plaster immobilization of the knee joint tested by us allows to create a persistent pronounced limitation of movements and can be used for further study of various methods of prevention and treatment of immobilization contractures.

#### **Conclusion**

Experimental model of 4-weeks plaster immobilization of the knee joint allows to create a persistent pronounced limitation of movements and can be used for further study of various methods of prevention and treatment of immobilization contractures.

In the future, it is planned to study various modes of vibration therapy for prevention and treatment of immobilization contractures in the experiment.

#### **DECLARATIONS:**

##### **Disclosure Statement**

The authors have no potential conflicts of interest to disclosure, including specific financial interests, relationships, and/or affiliations relevant to the subject matter or materials included.

##### **Data Transparency**

The data can be requested from the authors.

##### **Statement of Ethics**

The authors have no ethical conflicts to disclosure.

##### **Funding Sources**

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##### **Consent for publication**

All authors give their consent to publication.

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