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# Limb compression induces multi-system genetic damage in rats

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**Abstract**

Muscle crush injury is a common trauma in the modern society after as a result of mass disasters after penetration into muscle by high-velocity projectiles, blunt external trauma, or by gravity during prolonged immobilization in comatose patients after head trauma, alcoholic or drug overdose. However, the underlying mechanisms linking these alterations are still not fully understood, especially in acute phase. The aim of this study was to analyze genomic instability in multiple organs of rats after acute muscle injury by means of single cell gel (comet) assay. Rats were randomly distributed into three groups ($n = 6$ each group): control group and experimental groups: sacrificed 6 h as 12 h after muscle compression. These results indicate genetic damage in peripheral blood cells as depicted by tail moment results. DNA breakage was also detected in liver, lung and kidney cells after acute muscle injury for two times evaluated. Heart cells showed genetic damage after 12 h following muscle compression. Taken together, our results suggest that acute muscle injury induces genomic damage in multiple organs of Wistar rats. This novel finding offers new insights into the underlying mechanisms of the relationship between acute crush muscle injury and clinical manifestations that can occur during limb compression.


*Keywords:* Rat; Acute muscle injury; Genetic damage

## 1. Introduction

Muscle crush injury classically occurs in casualties of mass disasters and may also occur after penetration into muscle by high-velocity projectiles, blunt external trauma, or by gravity during prolonged immobilization in comatose patients after head trauma or alcoholic or drug overdose [1]. In the modern society, injuries to the musculoskeletal apparatus are difficult to treat and are often associated with pain, discomfort, reduced mobility, and limited quality of life [2]. Thus, muscle injuries have a high impact for medical system and need to be better analyzed as well as the systemic consequences.

The pathogenesis of all crush injuries includes rhabdomyolysis, whereby increased and prolonged pressure on skeletal muscle leads to cellular death and the release of its intracellular contents [3]. This can lead to systemic host response such as hyperkalaemia, acidosis, acute renal failure, and hypovolaemic shock, which are the main clinical manifestation of crush syndrome [2]. Herein, a crucial requirement for the development of novel therapeutic strategies after muscle injuries is to understand how living organisms are able to act for promoting muscle regeneration.

It is well established that DNA damage as well as cell mutation induces genetic instability through multiple pathways [4]. Accumulation of such abnormalities in the genome is associated with genomic instability as far as increased risk for several degenerative diseases [5]. Relevant genetic parameters include the detection of DNA-damage, accumulation and persistence of damage, which includes DNA repair and tissue regeneration. It is important to stress that the types of lesions and DNA repair are different among individuals and tissues; it appears important to study the damage in two or more target organs or tissues [6]. To the best of our knowledge, little is known about the genetic basis after acute muscle injury, particularly its consequences in multiple organs as a result of systemic host response.

To date, a variety of assays has been proposed for detecting genomic instability, including those that assess DNA

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damage, metaphase chromosomal aberrations, sister chromatid exchanges and micronucleus assay. The single cell gel (comet) assay, in the alkaline version, is a rapid, simple, and reliable biochemical method for evaluating DNA damage in mammalian cells [7]. This technique includes embedding cells in agarose gel on microscope slides and lysing with detergent and high salts. During electrophoresis under alkaline conditions, cells with damaged DNA display increased DNA migration resulting DNA strand breaks, alkalilabile, abasic sites, and incomplete repair sites toward anode. Broken DNA migrates farther in the electric field and the cell resembles a 'comet' with brightly fluorescent head and a tail region [8]. The extent of the comet is related to increased DNA damage. These images can be analyzed and compared in a cell-to-cell basis. Our research group has applied with success the single cell gel (comet) assay under different conditions and paradigms for studying several pathological conditions [9–12]. For this reason, a great deal of enthusiasm was raised by the application of the methodology for better understanding the biological basis of acute muscle injury using murine models.

Therefore, we used the single cell gel (comet) assay as a putative biomarker to predict genomic instability in multiple organs of rats suffering acute muscle injury. Certainly, such data will contribute to better understanding tissue alterations induced by acute muscle injury that contributes to the crush syndrome.

## 2. Material and methods

The experimental protocol was approved by our Institutional Committee of Ethics and followed the guidelines of the Guide for the Care and Use of Laboratory Animals (USA National Academy of Science).

### 2.1. Experimental procedures

Adult male Wistar rats, 250–300 g, $n = 18$, were fasted overnight from food but had free access to water 12 h before the experiment. Under general anesthesia (pentobarbital, intraperitoneal, 30 mg/kg weight plus 2% Xylazine hydrochloride im 5 mg/kg weight), the rats were submitted to tracheal intubation (polyethylene cannula, 1.7 mm inner diameter) and maintained in spontaneous breathing. After clinical stabilization, 12 animals were randomized into 2 experimental groups ($n = 6$) according to the time of muscle compression (6 h and 12 h) or control procedures ($n = 6$).

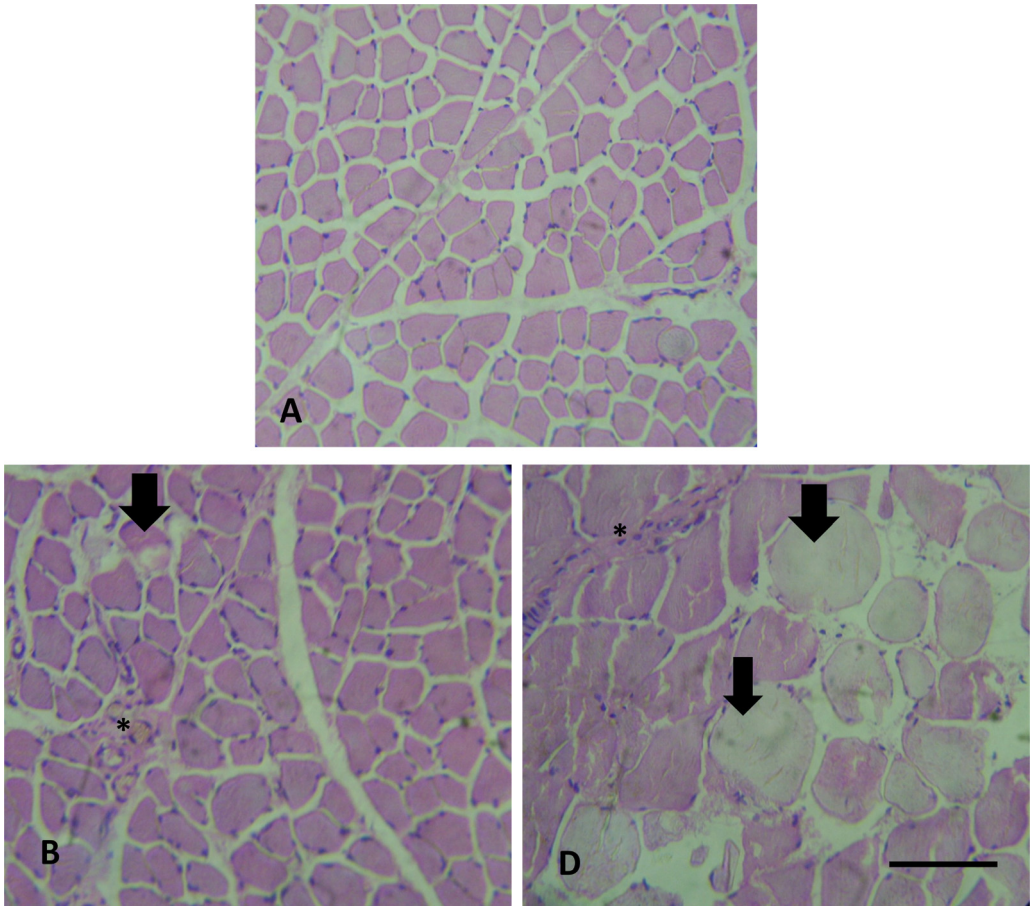


Fig. 1. Photomicrographies of muscle suffering crush injury. (A) Control; (B) after 6 h following compression and (C) after 12 h following compression 40× magnification. H.E. stain. Arrow: muscle degeneration and *inflammatory process.