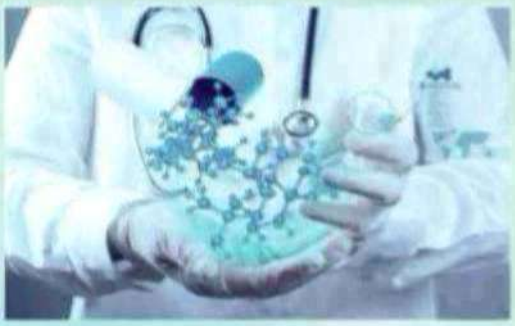
New Day in Medicine
Новый День в Медицине
NDM

TIBBIYOTDA YANGI KUN

Ilmiy referativ, marifiy-ma'naviy jurnal



AVICENNA-MED.UZ

ISSN 2181-712X.
EiSSN 2181-2187

8 (94) 2026

ТИББИЁТДА ЯНГИ КУН
НОВЫЙ ДЕНЬ В МЕДИЦИНЕ
NEW DAY IN MEDICINE

Илмий-рефератив, маънавий-маърифий журнал
Научно-реферативный,
духовно-просветительский журнал

УЧРЕДИТЕЛИ:

БУХАРСКИЙ ГОСУДАРСТВЕННЫЙ МЕДИЦИНСКИЙ ИНСТИТУТ
ООО «ТИББИЁТДА ЯНГИ КУН»

Национальный медицинский исследовательский центр хирургии имени А.В. Вишневского является генеральным научно-практическим консультантом редакции

Журнал был включен в список журнальных изданий, рецензируемых Высшей Аттестационной Комиссией Республики Узбекистан (Протокол № 201/03 от 30.12.2013 г.)

8 (94)

2026

Август

www.bsmi.uz
https://newdaymedicine.com
E: ndmuz@mail.ru
Тел: +99890 8061882

Received: 20.07.2026, Accepted: 06.08.2026, Published: 10.08.2026

UO‘K 612.172.6: 616.8-009.614:616-022.912-092.9

HISTOMORPHOLOGICAL CHANGES IN CARDIAC TISSUE UNDER THE INFLUENCE OF GENERAL ANESTHESIA FOLLOWING SOFT TISSUE INJURY OF THE LOWER LIMB IN OUTBRED WHITE RATS

Azamat Askarovich Turniyozov, https://orcid.org/0009-0007-5246-3698,
Xudoyberdiyev Dilshod Karimovich https://orcid.org/0009-0004-7508-7400

Bukhara State Medical Institute named after Abu Ali ibn Sina, Uzbekistan,
Bukhara, st. A. Navoi. 1 Tel: +998 (65) 223-00-50 e-mail: info@bsmi.uz

✓ ***Resume***

The analysis of morphological changes in the cardiac tissue following the effects of general anesthesia after experimental soft tissue injury of the right hind limb in 16-month-old outbred white rats demonstrated that this age group exhibited increased vascular permeability accompanied by plasmorrhagia, development of interstitial edema, and swelling of connective tissue fibers. Histochemical examination using toluidine blue staining revealed the accumulation of acidic mucopolysaccharides in the perivascular region. In addition, small lipid droplets were detected in the sarcoplasm of cardiomyocytes, a finding uncharacteristic of this age and indicative of the early onset of metabolic disturbances and reversible protein denaturation (dystrophic changes). Morphometric analysis of cardiac tissue following experimental injury and exposure to general anesthesia showed a 5.8% increase in the area occupied by coarse fibrous structures. The area occupied by blood vessels increased by 5.62%, reflecting vascular congestion as well as sclerotic and dystrophic changes in the vascular wall. Furthermore, the muscular component decreased by 6.36% compared with the control group due to the development of atrophic changes.

Keywords: outbred white rats, lower limb, soft tissue, injury, general anesthesia, cardiac tissue, histomorphological changes.

ГИСТОМОРФОЛОГИЧЕСКИЕ ИЗМЕНЕНИЯ В ТКАНИ СЕРДЦА ПОД ВЛИЯНИЕМ ОБЩЕЙ АНЕСТЕЗИИ ПОСЛЕ ТРАВМЫ МЯГКИХ ТКАНЕЙ НИЖНЕЙ КОНЕЧНОСТИ У БЕЛЫХ БЕСПОРОДНЫХ КРЫС

Турниёзов Азамат Аскарович, https://orcid.org/0009-0007-5246-3698,
Худойбердиев Дилшод Каримович https://orcid.org/0009-0004-7508-7400

Бухарский государственный медицинский институт имени Абу Али ибн Сины, Узбекистан, г. Бухара, ул. А. Навои. 1 Тел: +998 (65) 223-00-50 e-mail: info@bsmi.uz

✓ ***Резюме***

Анализ морфологических изменений в ткани сердца под воздействием общей анестезии после экспериментальной травмы мягких тканей правой задней конечности у 16-месячных белых беспородных крыс показал, что в данной возрастной группе наблюдалось повышение проницаемости кровеносных сосудов с развитием плазморрагии, выраженный интерстициальный отёк, а также набухание соединительнотканных волокон. При гистохимическом исследовании с использованием толуидинового синего в периваскулярной области выявлено накопление кислых мукополисахаридов. Кроме того, в саркоплазме кардиомиоцитов обнаружены мелкие жировые капли, что не характерно для данного возраста и свидетельствует о раннем развитии нарушений обмена веществ и обратимой денатурации белков (дистрофии). Морфометрическая оценка морфологических изменений ткани сердца после экспериментальной травмы и воздействия общей анестезии показала увеличение площади грубоволокнистых структур на 5,8%. Установлено также увеличение площади, занимаемой кровеносными сосудами, на 5,62% вследствие их полнокровия, а также склеротических и дистрофических изменений сосудистой стенки. В то же время площадь мышечного компонента вследствие атрофических изменений уменьшилась на 6,36% по сравнению с контрольной группой.

Ключевые слова: белые беспородные крысы, нижняя конечность, мягкие ткани, травма, общая анестезия, ткань сердца, гистоморфологические изменения.

OQ ZOTSIZ KALAMUSHLAR PASTKI MUCHASI YUMSHOQ TO‘QIMASI JAROHATIDAN KEYIN UMUMIY ANESTETIK TA’SIRINING YURAK TO‘QIMASIDAGI GISTOMORFOLOGIK O‘ZGARISHLARI

Turniyozov Azamat Askarovich, https://orcid.org/0009-0007-5246-3698,
Xudoyberdiyev Dilshod Karimovich https://orcid.org/0009-0004-7508-7400

Abu Ali ibn Sino nomidagi Buxoro davlat tibbiyot instituti, O‘zbekiston, Buxoro sh. A. Navoiy kochasi 1 Tel: +998 (65) 223-00-50 e-mail: info@bsmi.uz

✓ ***Rezyume***

Eksperimental 16 oylik oq zotsiz kalamushlar o‘ng orqa oyoq yuzasi yumshoq to‘qimasi jarohatidan keyin umumiy anestetik ta’sirining yurak to‘qimasidagi morfologik o‘zgarish tahlili shuni ko‘rsatadiki; 16 oylik guruhlarda qon-tomirlar o‘tkazuvchanligi ortib, plazmorragiya jarayoni rivojlanganligi, interstisial to‘qimaning shishi rivojlanib, biriktiruvchi tolalarda ham bo‘kish yuzaga kelib, gistokimyoviy bo‘yoqlarda, perivaskulyar sohada nordon mukopolisaxaridlar to‘planganligi toluidin bo‘yog‘i orqali aniqlanishi, bundan tashqari kardiomiotsitlar sarkoplazmasida mayda-mayda yog‘ tomchilari rivojlanganligi, bu yoshga xos bo‘lmagan, moddalar almashinuvi, oqsillarning qaytar denaturatsiyasi (distrofiya) ning erta boshlanganligi aniqlanildi. eksperimental jarohatidan keyin umumiy anestetik ta’sirining yurak to‘qimasidagi morfologik o‘zgarish morfometrik ko‘rsatkichlari asosida aksariyat, dag‘al tolali tuzilmalarni 5,8% ga ko‘payganligi aniqlandi. Qon-tomirlar bo‘shlig‘ida to‘laqonlik, devorida sklerotik va distrofik o‘zgarishlar natijasida egallagan maydon 5,62% ga oshganligi, mushak komponentlarida atrofik o‘zgarishlarning nazorat guruhiga nisbatan 6,36% ga kamayganligi aniqlandi.

Kalit so’zlar: oq zotsiz kalamushlar, pastki mucha, yumshoq to‘qima, jarohat, umumiy anestetik vositalar, yurak to‘qimasi, gistomorfologik o‘zgarishlar.

Research

To date, a wide range of modern analgesics and anesthetic agents has been developed. These drugs have significantly facilitated the performance of invasive procedures in surgery, dentistry, and traumatology. Although the effects of anesthetic techniques and pharmacological agents on various organs have been extensively investigated over many years, the morphological changes induced by anesthetics in cardiac tissue have not yet been fully elucidated.

Cardiovascular diseases (CVDs) remain one of the leading global health challenges. According to the latest Global Burden of Disease (GBD) report, approximately 19.2 million people died from cardiovascular diseases in 2023, accounting for nearly one in every three deaths worldwide. This figure has increased substantially compared with 1990, and cardiovascular diseases continue to be the leading cause of mortality. The majority of deaths are attributable to ischemic heart disease (coronary artery disease) and stroke. In 2023, more than 240 million individuals were estimated to be living with ischemic heart disease. Furthermore, modifiable risk factors—including obesity, hypertension, diabetes mellitus, unhealthy diet, and air pollution—play a significant role in the development of cardiovascular diseases [1].

Heart failure is a clinical syndrome resulting from decompensated myocardial dysfunction. It is characterized by increased interstitial fluid accumulation and reduced perfusion of organs and tissues. The underlying pathophysiological mechanism is the inability of the heart to maintain sufficient cardiac output to meet the metabolic demands of the body, or its ability to do so only by increasing ventricular end-diastolic pressure. In some patients with heart failure, clinical manifestations develop due to impaired ventricular filling or emptying associated with compromised cardiac pump function. Myocardial dysfunction, whether systolic or diastolic, is initially asymptomatic and only later progresses to clinically evident heart failure [2].

The distribution of certain anesthetic agents is limited. Non-depolarizing neuromuscular blocking agents are polar compounds with poor lipid solubility and relatively high molecular weight. Consequently, their distribution is largely confined to the extracellular compartment, as they cannot readily cross cell membranes. Nevertheless, their extracellular pharmacological activity has been well established [3].

Neuromuscular transmission monitoring is routinely performed in patients receiving neuromuscular blocking agents and is particularly valuable during regional and local anesthesia for assessing the degree of sensory blockade. This technique is based on electrical stimulation of a peripheral nerve followed by recording the resulting muscle contraction. In anesthesiology, the ulnar nerve is most commonly stimulated, and the contraction force of the adductor pollicis muscle is measured [4].

Muscle tissue injury associated with infiltration anesthesia is primarily attributed to an increase in extracellular calcium (Ca^{2+}) concentration. Among local anesthetics, bupivacaine exhibits the greatest myotoxic potential, followed by ropivacaine, lidocaine, and tetracaine. Recent studies have also focused on the effects of anesthetics on mitochondrial ATP metabolism. Moreover, anesthetic agents affect cardiac sodium (Na^{+}) channels, leading to delayed ion dissociation. Certain pharmacological properties of anesthetics are associated with the accumulation of their R-enantiomers. Some anesthetic agents also promote myocardial relaxation, improve tissue perfusion, and reduce skeletal muscle tone [5].

An increase in intracellular calcium concentration activates calcium-dependent processes within the sarcoplasm and may alter intracellular pH by stimulating acid hydrolases. These changes can initiate cellular autolysis ("self-digestion"), in which the plasma membrane is damaged first, resulting in the release of intracellular enzymes into the bloodstream. The interaction of local anesthetics with membrane receptors, including those located on the sarcoplasmic reticulum, may induce excessive release of Ca^{2+} from intracellular calcium stores. Conversely, protonated forms of local anesthetics inhibit calcium release from the sarcoplasmic reticulum. Other investigators have suggested that the myotoxic effects of local anesthetics are associated with impaired mitochondrial oxidative phosphorylation, leading to the development of oxidative stress within muscle cells [6].

Aim of the Study

The aim of the present experimental study was to determine the morphological and morphometric changes in the cardiac tissue of 16-month-old outbred white rats following the administration of general anesthesia after experimental soft tissue injury of the right hind limb.

Materials and Methods

A total of 150 outbred white rats of both sexes, aged 3, 9, and 16 months, were included in the study. All laboratory animals were housed in the same vivarium under standardized environmental conditions. The animals had unrestricted access to drinking water and were maintained on a balanced diet. Particular attention was paid to proper animal care and husbandry throughout the preparation and conduct of the experiment. Feeding schedules, dietary consistency, and hygiene standards were strictly maintained.

Throughout the experimental period, animals that died were buried, and carcasses were disinfected with a 20% chlorine solution in accordance with the institutional protocol for laboratory animal disposal.

The experimental animals were divided into three groups:

Group I (Control group): Intact outbred white rats (n = 10) maintained on a standard laboratory diet and used as the control group for comparison with the experimental groups.

Group II: Outbred white rats (n = 70) subjected to experimental soft tissue injury of the right hind limb followed by administration of a local anesthetic. Morphological evaluation was performed 60 minutes after anesthesia.

Group III: Outbred white rats (n = 70) subjected to the same experimental injury followed by administration of a general anesthetic, with tissue examination performed 120 minutes after anesthesia.

Experimental mechanical injury was induced in 120 rats aged 3, 9, and 16 months. Mechanical trauma was produced in the soft tissues of the right hind limb, resulting in localized soft tissue injury. Following the procedure, the animals were housed in separate cages according to age and experimental group.

In the second experimental group, a 10% lidocaine spray (10 mL) was used as the local anesthetic. The preparation was applied directly to the injured soft tissue of the right hind limb.

Lidocaine is characterized by its rapid onset of action and intermediate duration of anesthetic effect. Therefore, it is widely used for infiltration, regional blockade, and topical anesthesia. In some

clinical situations, long-acting anesthetics such as bupivacaine are preferred for spinal and epidural anesthesia; however, lidocaine provides a more rapid onset of action. The addition of epinephrine, a vasoconstrictor, reduces local bleeding and delays systemic absorption of lidocaine, thereby almost doubling the duration of anesthesia. Lidocaine acts by blocking voltage-gated sodium channels, preventing the generation and propagation of nerve impulses along sensory nerve fibers. Consequently, it suppresses not only pain transmission but also other sensory modalities. Compared with procaine, the anesthetic effect of lidocaine is 2–6 times more potent, with a duration of action of up to 75 minutes, extending to more than 2 hours when combined with epinephrine. When administered locally, lidocaine also produces vasodilation.

The next stage of the experiment was conducted 60 minutes after administration of the local anesthetic.

In the third experimental group, general anesthesia was administered following experimental soft tissue injury of the right hind limb. Nitrous oxide (N_2O) was used as the general anesthetic and was administered by inhalation.

Nitrous oxide is a colorless, odorless gas with a slightly sweet taste. It is readily soluble in water, blood, and tissue fluids and is eliminated unchanged through the respiratory tract. It is supplied in liquefied form in gray steel cylinders, with a gas pressure of approximately 50 atmospheres. In clinical practice, nitrous oxide is commonly used as a component of balanced general anesthesia in combination with analgesics, muscle relaxants, neuroleptics, and other anesthetic agents. It is administered exclusively in combination with oxygen, and the oxygen concentration in the inhaled gas mixture should not be less than 20%. Because of its relatively low anesthetic potency, even an 80% nitrous oxide–20% oxygen mixture produces mainly analgesia rather than complete surgical anesthesia. Nitrous oxide is nonflammable and nonexplosive, has a rapid onset and recovery, may cause nausea and vomiting, and does not irritate the respiratory tract. However, it is a relatively weak anesthetic, does not provide sufficient skeletal muscle relaxation, may lead to diffusion hypoxia, and prolonged administration may result in bone marrow suppression.

The subsequent stage of the experiment was carried out 120 minutes after administration of the general anesthetic.

Continuation of the Materials and Methods

At the predetermined experimental endpoints, the animals were euthanized by decapitation in accordance with the study protocol.

Following laparotomy, the heart was carefully excised. The transverse diameter (width) of the heart was measured using a millimeter ruler.

The study employed organometric, histomorphometric, microscopic, and statistical methods.

The principal study specimens consisted of 0.5 × 5 cm tissue samples obtained from different regions of the hearts of outbred white rats. The preparation of histological specimens was carried out over a six-day protocol, as described below.

Histological Processing

Day 1. Two tissue specimens were obtained from each biopsy sample, labeled, wrapped in gauze or placed in tissue cassettes, and fixed in 10% neutral buffered formalin (12% formalin solution, equivalent to 10% formaldehyde for animal tissues) for 24 hours. An additional portion of each specimen was archived. The specimen size, color, and tissue consistency were recorded on the laboratory requisition form.

Day 2. Following fixation, the tissue samples were removed from formalin and rinsed under running water for 1 hour to eliminate residual fixative. Tissue dehydration was subsequently performed using ascending concentrations of ethanol (80%, 90%, 96%, and 100%). After dehydration, the specimens were maintained at room temperature before further processing.

Day 3. For tissue clearing, absolute ethanol (100%) and o-xylene were mixed in a 1:1 ratio, and the specimens were immersed in this solution, followed by immersion in pure o-xylene. The cleared tissues were then transferred to a thermostat containing an equal mixture of o-xylene and paraffin at 37°C, followed by infiltration in molten paraffin at 56°C. The paraffin-impregnated tissues were subsequently embedded in wooden molds.

Day 4. The embedded paraffin blocks were labeled and mounted in Petri dishes or special metal embedding molds. Warm paraffin was poured over the tissue specimens to complete embedding. After solidification, the paraffin blocks were trimmed, labeled, and stored under refrigerated conditions to ensure adequate hardening before sectioning with a microtome.

Day 5. Paraffin blocks were sectioned using a microtome. The sections were mounted onto glass slides coated with a 1:1 mixture of protein adhesive and glycerol, then dried in a thermostat at 37°C. Each slide was assigned a registration number for identification.

Day 6. Histological sections were stained and examined using an **HL-19 trinocular microscope** equipped with specialized image analysis software for biological microscopy. Histological dyes were

selected according to their chemical properties and tissue affinity, including plant-derived dyes (hematoxylin), animal-derived dyes (carmine), and synthetic dyes (eosin), as well as acidic, basic, and neutral stains.

Histochemical Staining

Toluidine blue is a basic thiazine metachromatic dye with high affinity for acidic tissue components and therefore selectively stains tissues rich in DNA and RNA. Owing to its metachromatic properties, it is widely used as a special histochemical stain. Toluidine blue (also known as tolonium chloride) selectively binds to acidic tissue constituents, including sulfates, carboxylates, and phosphate radicals. Because of its affinity for nucleic acids, it strongly stains cell nuclei containing high concentrations of DNA and RNA. Toluidine blue has also been extensively used for detecting mucosal lesions and identifying specific tissue components through metachromasia. In the present study, it was employed for histochemical evaluation of cardiac tissue.

Results

The heart weight of 16-month-old outbred white rats ranged from 17.12 g to 26.03 g, with a mean value of 23.15 ± 1.10 g.

Histological examination demonstrated pronounced reparative changes in the cardiac tissue. Thickening of the endothelial cell layer was observed together with the presence of a fibrous connective tissue layer in the subendocardial region, characterized by abundant irregularly arranged connective tissue fibers. Bundles of this fibrous tissue extended between adjacent myocardial fibers.

Marked proliferative activity of stromal mesenchymal cells was observed, accompanied by increased synthesis of tropocollagen by fibroblasts, which subsequently resulted in the formation of mature collagen fibers. These changes led to **interposition of fibrous connective tissue between cardiomyocytes, disrupting the normal continuity and architecture of myocardial muscle fibers.

The myocardium also demonstrated varying degrees of pyknosis of cardiomyocyte nuclei, progressive destruction of myofibrils, and accumulation of large lipid droplets within the sarcoplasm. In addition, the interstitial capillaries were deformed and exhibited irregular vascular congestion. Focal areas consistent with sludge phenomenon (pseudo-stasis) were identified, while the vascular walls showed myxoid (mucoid) swelling, indicating degenerative alterations of the microvasculature (Figure 1).

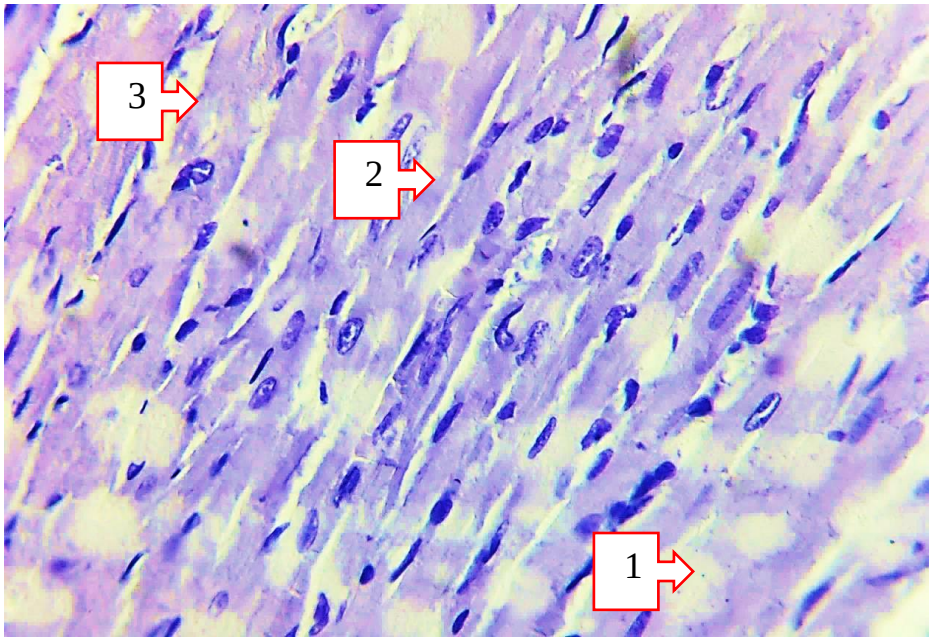


Figure 1. Morphological changes in the cardiac tissue of 16-month-old outbred white rats following the administration of general anesthesia after experimental soft tissue injury of the right hind limb.

Toluidine blue staining. Objective ×10, ocular ×10. 1. Large lipid droplets of various shapes and sizes in the sarcoplasm of cardiomyocytes (unstained). 2. Pyknotic (shrunken) nuclei of cardiomyocytes. 3. Marked proliferation of connective tissue fibers within the interstitial tissue.

The diameter of cardiomyocytes ranged from 22.11 μm to 29.13 μm, with a mean value of 26.74 ± 1.78 μm. The diameter of cardiomyocyte nuclei ranged from 12.75 ± 2.04 μm to 18.52 ± 1.05 μm, with a mean value of 14.84 ± 1.12 μm. Morphometric analysis demonstrated that cardiomyocyte nuclei occupied 20.45 ± 2.21% of the examined area, whereas the sarcoplasm–sarcomere complex accounted for 79.55 ± 1.05%. Histological examination of the myocardial vasculature revealed vascular congestion, stasis, thickening of vascular walls, dilation of the vascular lumen, increased vascular permeability, and plasmorrhagia. The total vascular area measured 5742.15 ± 105.5 arbitrary units. Morphometric analysis showed that, in the experimental group, the myocardial parenchyma occupied 74.40% to 81.23% of the examined area, with a mean value of 77.11 ± 1.52%. The myocardial interstitium—including connective tissue fibers, hypertrophied fibroblasts, and thickened vascular walls—occupied 19.77% to 25.60%, with a mean value of 22.89 ± 1.24% (Figure 3.5.12). The morphometric parameters of this experimental group in comparison with the control group are presented in Table 1.

Table 1.

Morphometric parameters of cardiac tissue in the control group and in 16-month-old outbred white rats following the administration of general anesthesia after experimental soft tissue injury of the right hind limb

№	Parameters (Mean)	16-month-old Control Group	16-month-old General Anesthesia Group
1	Heart weight (g)	15,45±5,10**	23,15±1,10*
2	Parenchyma (%)	82,32±2,15	77,4±452
3	Stroma (%)	17,88±2,54	22,89±1,23**
4	Cardiomyocyte diameter (μm)	25,25±2,22	26,74±1,78
5	Cardiomyocyte nuclear diameter (μm)	17,25±2,10*	14,84±1,12**
6	Cardiomyocyte nuclei (%)	22,12±2,15*	20,45±2,21**
7	Sarcoplasm–sarcomere area (%)	77,88±2,75	79,55±1,05
8	Blood vessel area (μm²)	5433,31±118 ,2	5742,15±105,5

Note: Statistically significant differences compared with the control group are indicated by asterisks ():* $p < 0.05$, $p < 0.01$, and $p < 0.001$, representing the level of significance between the mean values.

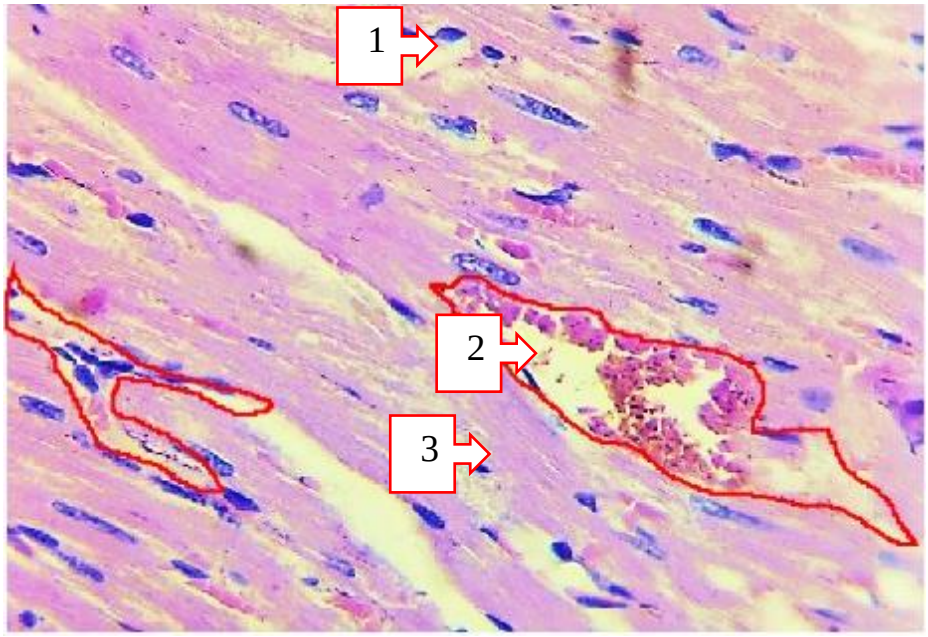


Figure 2. Morphological changes in the cardiac tissue of 16-month-old outbred white rats following the administration of general anesthesia after experimental soft tissue injury of the right hind limb.

Toluidine blue staining. Objective ×10, ocular ×10. 1. Proliferation of connective tissue fibers within the myocardial interstitium. 2. Congested blood vessels with pseudo-stasis and dilatation of the vascular lumen. 3. Increased accumulation of large lipid droplets in the sarcoplasm of cardiomyocytes.

Histological examination of cardiac tissue stained by the Van Gieson method demonstrated that the myocardial stroma was extensively replaced by coarse, densely packed collagen bundles staining bright red, instead of the normal fine connective tissue fibers. These collagen fibers exhibited irregular and disorganized growth throughout the stroma.

These findings indicate a marked increase in the proliferative activity of stromal mesenchymal cells, accompanied by enhanced tropocollagen synthesis by fibroblasts, resulting in progressive collagen deposition and myocardial fibrosis. Only a small number of intact cardiomyocytes remained between the homogenized red-stained collagen bundles, appearing yellowish-golden after Van Gieson staining. Cardiomyocyte nuclei varied considerably in size and staining intensity, ranging from lightly to intensely stained, and were distributed asymmetrically. Accelerated interposition of connective tissue was observed, characterized by the ingrowth of zigzag (step-like) collagen fibers between adjacent cardiomyocytes, progressively replacing normal myocardial fibers. Macroscopic examination of the heart revealed marked structural remodeling of the ventricular wall. Compared with the normal conical shape, the heart appeared rounded, with evident ventricular hypertrophy, enlargement of the cardiac circumference, and transformation into a more quadrangular configuration (Figure 3).

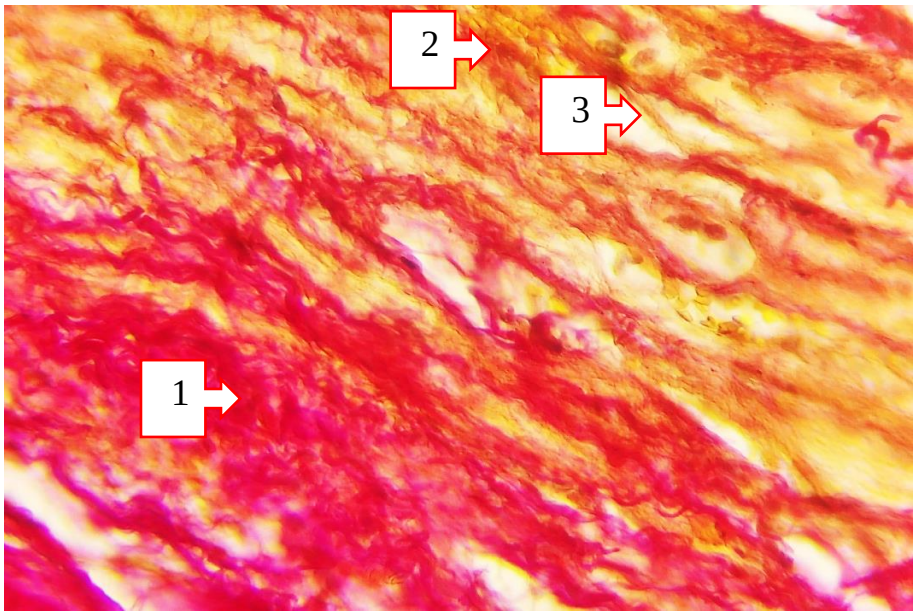


Figure 3. Morphological changes in the cardiac tissue of 16-month-old outbred white rats following the administration of general anesthesia after experimental soft tissue injury of the right hind limb.

Van Gieson stain. Objective ×10, ocular ×10. 1. Marked focal thickening of subendocardial elastic collagen fibers, stained dark pink. 2. Extensive spread of elastic collagen fibers between cardiomyocytes. 3. Clear spaces within the interstitial tissue, indicative of edema.

Histological examination of cardiac tissue stained with the Van Gieson (picrofuchsin) method demonstrated that collagen fibers beneath the endocardium of the ventricular wall were considerably more prominent than those in the middle layers of the subendocardial tissue. The connective tissue exhibited pronounced edema, while connective tissue cells showed increased homogenization. In several areas, collagen fibers stained an intense dark red in a focal pattern. These red-stained collagen fibers extended deeply into the myocardium, where they appeared as homogenized bundles with varying shades of dark red to crimson, displaying a characteristic zigzag arrangement.

Intact cardiomyocytes stained yellow-gold and were surrounded by abundant red-stained collagen fibers, indicating progressive myocardial fibrosis. Fibrous connective tissue bundles were closely apposed to the myocardial layer. The adjacent striated muscle fibers were thickened due to cardiomyocyte hypertrophy, and their nuclei were correspondingly enlarged.

Relatively large interstitial cavities were observed in some regions adjacent to the connective tissue. These spaces were associated with the presence of lipid droplets within the sarcoplasm of cardiomyocytes. In addition, myocardial fibers appeared deformed and irregularly arranged in several areas. The interstitial space was variably expanded, accompanied by proliferation of connective tissue cells, which stained pink with the Van Gieson technique. Cardiomyocyte nuclei exhibited considerable variation in size and staining intensity, ranging from lightly stained to intensely stained nuclei (Figure 4).

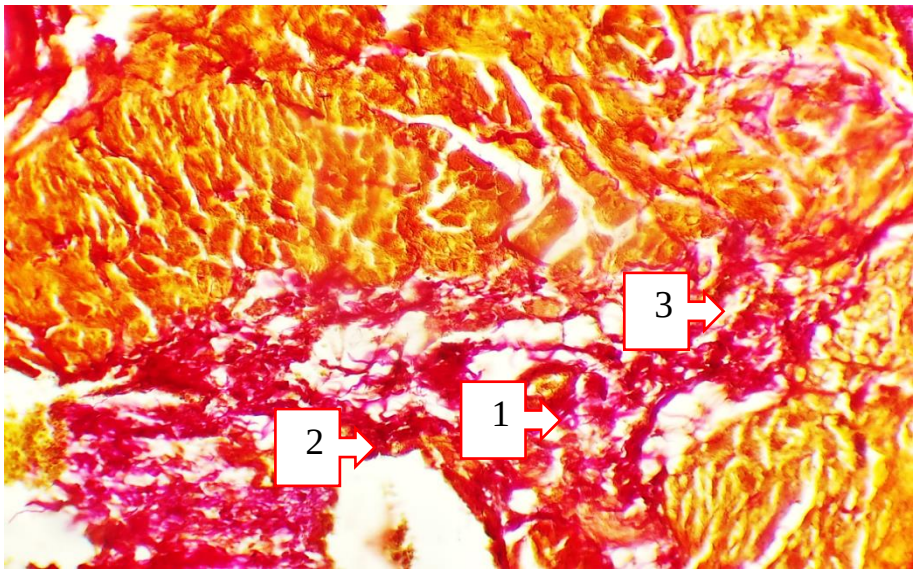


Figure 4. Morphological changes in the cardiac tissue of 16-month-old outbred white rats following the administration of general anesthesia after experimental soft tissue injury of the right hind limb.

Van Gieson stain. Objective ×10, ocular ×10. 1. Thickening and sclerosis of the blood vessel wall. 2. Marked focal thickening of elastic fibers in the perivascular region, stained dark pink. 3. Extensive distribution of elastic collagen fibers between cardiomyocytes.

When the cardiac tissue was stained with toluidine blue, pronounced accumulation of acidic glycosaminoglycans (GAGs) was observed in the myocardial stroma, appearing as an intense blue coloration. The increased staining intensity indicates the development of acute tissue hypoxia, accompanied by a marked reduction in vascular perfusion of the interstitial tissue. The blood vessel walls exhibited a darker blue staining than the surrounding tissues, representing the characteristic morphological and histochemical manifestations of hypoxic injury (Figure 5).

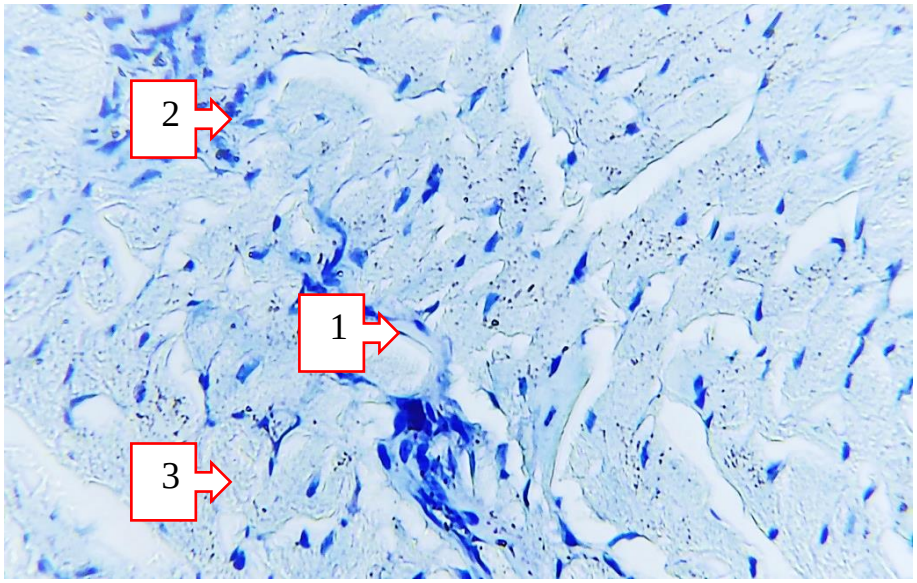


Figure 5. Morphological changes in the cardiac tissue of 16-month-old outbred white rats following the administration of general anesthesia after experimental soft tissue injury of the right hind limb.

Toluidine blue stain. Objective ×10, ocular ×10. 1. Thickening of the blood vessel wall and perivascular region in the subendocardial area, intensely stained dark blue. 2. Focal thickening of elastic fibers within the interstitial tissue, stained dark blue. 3. Cardiomyocyte nuclei stained dark blue, whereas the sarcoplasm exhibited a pale blue appearance due to abundant intracellular lipid droplets.

Histochemical examination of cardiac tissue demonstrated a progressive accumulation of acidic mucopolysaccharides (glycosaminoglycans) throughout the stromal compartment. Such alterations may interfere with the normal rhythmic contractility of the myocardium. Prolonged impairment of myocardial perfusion was associated with the development of secondary structural changes within the cardiac wall, particularly along the atrioventricular (Aschoff–Tawara) node and the bundle of His, where fibrotic tissue composed of coarse connective tissue fibers was observed. The formation of collagen-rich, irregularly oriented fibrous structures resulted in isolated islands of surviving cardiomyocytes, whose contraction became asynchronous. Morphologically, these findings are characteristic of cardiosclerosis.

To further confirm the above morphological observations, cardiac tissue from 16-month-old control rats was stained using the Periodic Acid–Schiff (PAS) reaction. Compared with the 3-month-old control group, age-related histochemical changes were observed. Over time, intensely PAS-positive structures developed predominantly around blood vessels that continuously supply the myocardium, appearing as dark red deposits, whereas the remaining cardiomyocytes displayed a relatively uniform pink-red staining pattern.

Under normal conditions, the PAS reaction stains neutral mucopolysaccharides a pink-red color. These findings suggest that, even under physiological aging conditions, neutral mucopolysaccharides are gradually replaced by acidic mucopolysaccharides within the cardiac tissue (Figure 6).

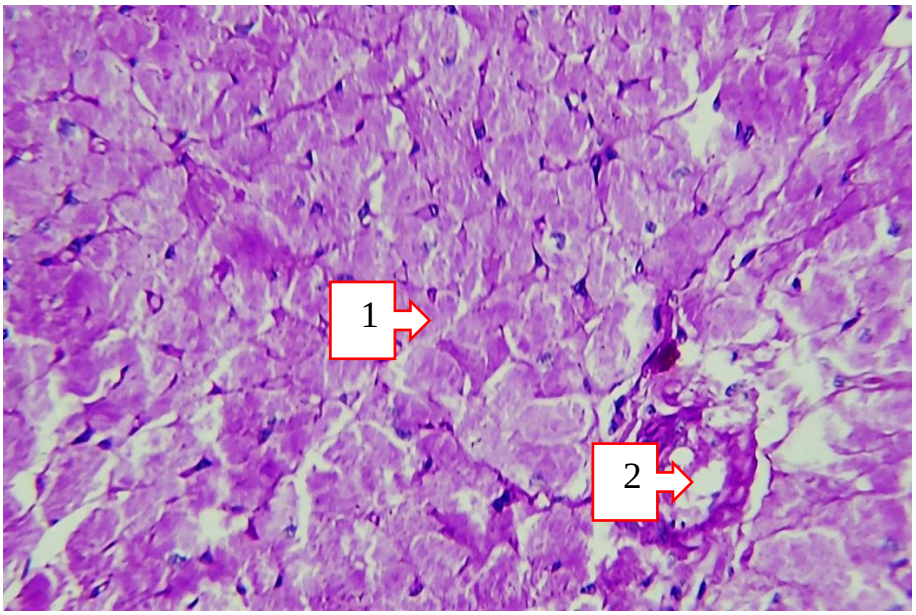


Figure 6. Cardiac tissue morphology in 16-month-old control outbred white rats following experimental soft tissue injury of the right hind limb and exposure to general anesthesia.

Periodic Acid–Schiff (PAS) stain. Objective ×10, ocular ×10. 1. Cardiomyocyte sarcoplasm stained pink-red. 2. Thickened blood vessel wall and interstitial connective tissue stained dark pink-red.

Histochemical examination of the cardiac tissue of 16-month-old experimental outbred white rats using the Periodic Acid–Schiff (PAS) stain demonstrated an uneven distribution of acidic mucopolysaccharides throughout the myocardium. Both myocardial fibers and connective tissue exhibited a uniform pale pink staining pattern, while intracellular lipid droplets appeared as sharply demarcated unstained (colorless) areas.

These findings indicate disturbances in metabolic processes within both the myocardial parenchyma and stromal components of the heart. In addition, the results suggest that neutral mucopolysaccharides were progressively replaced by acidic mucopolysaccharides, which accumulated within the cardiac tissue during the pathological process (Figure 7).

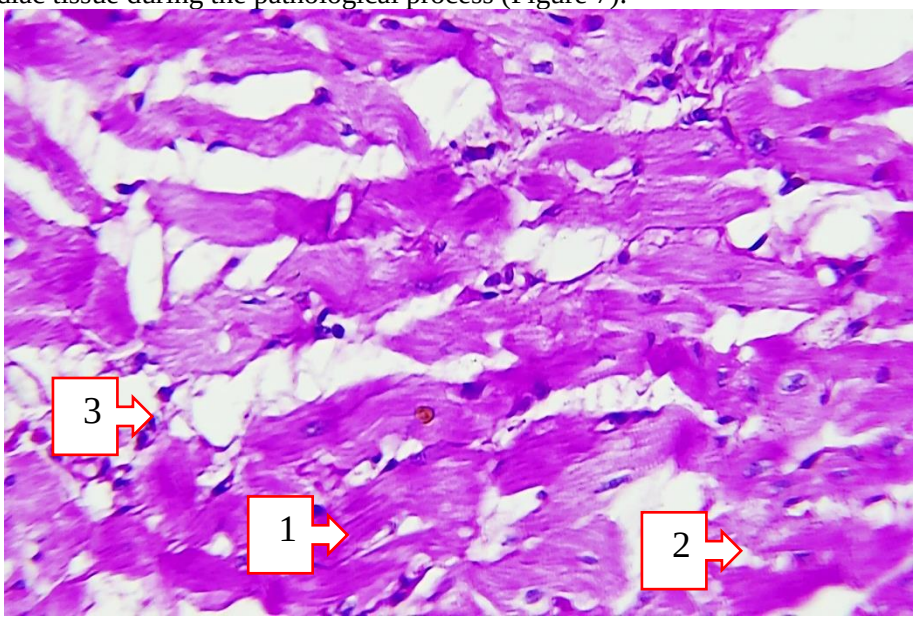


Figure 7. Morphological changes in the cardiac tissue of 16-month-old outbred white rats in the experimental group following general anesthesia after experimental soft tissue injury of the right hind limb.

Periodic Acid–Schiff (PAS) stain. Objective ×10, ocular ×10. 1. Cardiomyocytes stained pale pink. 2. Focal accumulation of lipid droplets within the sarcoplasm of cardiomyocytes. 3. Large focal lipid deposits in the interstitial tissue accompanied by proliferation of connective tissue fibers.

Conclusion

The analysis of morphological changes in the cardiac tissue of 16-month-old outbred white rats following the administration of general anesthesia after experimental soft tissue injury of the right hind limb demonstrated that vascular permeability was significantly increased, resulting in the development of plasmorrhagia. Marked interstitial edema was observed together with swelling of connective tissue fibers. Histochemical examination using toluidine blue staining revealed the accumulation of acidic mucopolysaccharides within the perivascular region. In addition, numerous small lipid droplets were identified in the sarcoplasm of cardiomyocytes. These findings are not characteristic of normal aging and indicate the early development of metabolic disturbances and reversible protein denaturation (dystrophic changes).

Microscopic examination of the cardiac tissue in the experimental group of 16-month-old outbred white rats revealed the following major pathological alterations:

1. Destructive changes. Marked proliferative activity of mesenchymal cells was observed in the subendocardial region, accompanied by increased tropocollagen synthesis by fibroblasts. This process subsequently resulted in the formation of collagen fibers and the development of interposition of connective tissue between cardiomyocytes, whereby coarse fibrous connective tissue infiltrated the spaces between myocardial fibers, disrupting their normal architecture and continuity. These alterations indicate progressive myocardial sclerosis, ultimately predisposing to cardiomyocyte atrophy and cell death.

2. Metabolic disturbances. Cardiomyocytes exhibited varying degrees of nuclear pyknosis, progressive destruction of myofibrils, and the accumulation of large lipid droplets within the sarcoplasm. These changes represent characteristic morphological features of severe impairment of myocardial metabolism.

Overall, analysis of the morphological and morphometric findings in the cardiac tissue of 16-month-old outbred white rats, summarized in Table 1, demonstrated that experimental soft tissue

injury followed by general anesthesia induced significant structural remodeling of the myocardium. Morphometric analysis revealed a 5.8% increase in the area occupied by coarse fibrous connective tissue. The area occupied by blood vessels increased by 5.62% as a consequence of vascular congestion together with sclerotic and dystrophic alterations of the vascular wall. In contrast, the muscular component of the myocardium showed a 6.36% decrease compared with the control group, reflecting the development of atrophic changes.

LIST OF REFERENCES:

1. Барминский А. В., Егоров А. Н., Киров М. Ю. Методики регионарной анальгезии при абдоминальных вмешательствах в педиатрии // Вестник анестезиологии и реаниматологии. 2024:21(1):110–117.
2. Васильков В. Г. и др. Опыт применения различных методик регионарной анестезии в хирургии и травматологии // Медицинский алфавит. 2017:2(17):30–34.
3. Гурова С.В., Миндарь М.В., Ходакова Д.В. Современные аспекты в области анестезии мелких лабораторных животных // Южно–российский онкологический журнал. 2022:3:55–63.
4. Заболотских И. Б. и др. Прогнозирование кардиального риска в абдоминальной хирургии: результаты наблюдательного многоцентрового исследования // Комплексные проблемы сердечно–сосудистых заболеваний. 2024:13(2):176–195.
5. Королев М. В. и др. Анестезия при артроскопических операциях на нижних конечностях у пожилых пациентов // Инновационная медицина Кубани. 2020:4(20):65–72.
6. Лутай Ю. А. и др. Основные подходы к снижению периоперационных кардиальных осложнений при внесердечных хирургических вмешательствах //Крымский терапевтический журнал. 2023:1:40–46.

Entered 20.07.2026