ORIGINAL ARTICLE

CONTENTS

Morphometric analysis of the morphological state of skin vascular plexuses under opioid in an experiment

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ABSTRACT

Aim: The patterns of qualitative and quantitative changes in the microvascular network in the skin of adult male white rats under of nalbuphine administration.
Materials and Methods: The research employed methods such as vascular bed injection, skin section clearing, and microscopic imaging using an MBI-1 microscope. Morphometric analysis of the microcirculation vessels was conducted, and statistical processing of the results was performed using specialized software.
Results: The nalbuphine significantly impacted the morphological state of the white rat's skin after two weeks of administration, with the initial changes occurring in the blood vessel plexuses. In the injected skin samples, both arterioles and capillaries were dilated, with the diameter of the subpapillary arteriolar network significantly increasing to 28.62±1.07 μm (control – 22.24±0.73 μm), and the diameter of intrapapillary capillary loops expanding to 6.20±0.11 μm (control – 5.91±0.26 μm). Arterioles exhibited tortuosity. After four weeks, the loops of the vascular plexus lost their delicate, lace-like structure, with microaneurysms in arterioles and sacculations in venules becoming evident. After six weeks of opioid exposure, significant structural alterations were observed in the blood vessels of the skin. Capillaries became obliterated, with some destroyed, and this process was accompanied by hemorrhages. The density of intrapapillary capillary loops decreased significantly to 59.0±2.0 (control – 79.60±2.078), while the trophic activity index increased to 39.490±1.307 μm (control – 27.172±1.143 μm),
Conclusions: Morphometric analysis of the morphological state of the vascular plexuses in the skin clearly illustrates the relationship between quantitative and qualitative changes in the structural organization of the microcirculation network under opioid exposure.

KEY WORDS: skin, vessels, morphometry, opioid

Wiad Lek. 2025;78(6):1125-1132. doi: 10.36740/WLek/204796 DOI

INTRODUCTION

Nowadays, medicine, unfortunately, cannot function without the use of opiates and opioids for therapeutic purposes [1-5]. Research on the effects of opioids on organ structure is scarce and contradictory [6=11]. Individuals who are forced to use opioids for extended periods have an increased mortality rate from cardiovascular diseases and a higher likelihood of fatal outcomes due to liver and kidney failure. Besides, regular use of narcotic substances severely damages the immune system [12]. In recent years, the number of inflammatory conditions among injection drug users has increased worldwide [13]. The continuous rise in the number of drug addicts in Ukraine has led to a series of new social, economic and medical problems [14-16].

The skin is the organ that responds first to exogenous and endogenous influences [17, 18]. In the event of any skin damage, the vascular plexuses of the skin are the first to react. Despite the relevance and importance of studying morphological changes in microcirculation network under pathological conditions, the professional literature lacks sufficient information on the vascular network of the skin, and information on its morphometric analysis under both normal physiological conditions and different pathological processes is nearly nonexistent.

The utilization of experimental animals, specifically white rats, for modeling opioid effects on the skin enables the investigation of dynamic structural alterations in the cutaneous microvasculature under prolonged exposure to the narcotic agent. The rationale for using this laboratory animal is the similarity between the skin structure of rats and humans.

AIM

To establish the patterns of qualitative and quantitative changes in the components of the cutaneous microcirculation bed in sexually mature male white rats under different durations of nalbuphine administration.

MATERIALS AND METHODS

The study was conducted on 40 sexually mature male white rats with an initial body weight ranging from 160 to 180 g and an age of 3.0 months

The study was conducted on 40 sexually mature male white rats with an initial body weight ranging from 160 to 180 g and an age of 3.0 months.

The animals selected for the study underwent thorough screening, including clinical examination, weighing, and marking. All animals were housed under standard vivarium conditions at Danylo Halytsky Lviv National Medical University. The experiments were conducted in compliance with ethical guidelines for the humane treatment of laboratory animals, as stipulated by the Law of Ukraine "On the Protection of Animals from Cruelty" (No. 3447-IV, 21.02.2006) and the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 18.03.1986).

The study material consisted of skin specimens from white rats with an injected vascular network.

The research utilized the following methodologies: injection of the skin vascular network in white rats, tissue section clearing and subsequent microscopic imaging using an MBI-1 microscope. Morphometric analysis of the microcirculation components of the skin was conducted. Statistical analysis of the research data was performed using specialized software. Furthermore, a model for prolonged opioid exposure in white rats was established and implemented.

The animals were euthanized by intraperitoneal administration of sodium thiopental at a dosage of 2.5 mg/100 g (25 mg/kg) of body weight.

After opening the ventral wall of the abdominal and thoracic cavities, clamps were applied to the intercostal arteries. A glass cannula with a rubber tip was inserted into the thoracic aorta, connected to a syringe, and secured with a silk ligature. The injection mass was then introduced into the vascular system via the syringe. The blood vessels of the rat's skin were filled with an ink-gelatin injection mass. The injection mass was prepared as follows: 100 g of gelatin was poured into 1 L of cold water and left for 24 hours. After the gelatin swelled, the mixture was heated in a water bath and filtered through several layers of gauze. The warm gelatin was then mixed with equal volumes of sodium citrate solution and ink before being injected into the arterial system of a freshly euthanized white rat. The volume of the mixture used for filling the arterial system ranged from 10 to 15 mL. After the injection, a ligature was applied to the abdominal aorta, and the skin from the gluteal region was collected for further study. This injection technique is technically simple and does not require scarce reagents. Moreover, it allows for the injection of both large vessels and microcirculation components, enabling differentiation of arterial, capillary, and venous segments.

The day after the injection with the ink-gelatin mass, the skin was immersed for two days in a 1:1 mixture of glycerin and ethanol. It was then cleared and stored in chemically pure glycerin.

After clearing the specimens, the segments of the skin vascular network were photographed in transmitted light using an MBI-1 microscope at magnifications of ×80 (objective ×10, eyepiece ×8) and ×160 (objective ×20, eyepiece ×8) with an Olympus FE 210 digital camera.

The study of the microcirculatory vessels in injected and cleared specimens allows for a reliable assessment of key quantitative parameters, including the diameter of microvessels, the density of the capillary network (DCN), and the area of trophic tissue activity (ATTA).

For the morphometric analysis of the angioarchitecture of white rat's skin, measurements of the diameters of arterioles, capillaries, and venules were performed. The vessel diameters were measured using an ocular micrometer. These measurements were conducted on cleared skin specimens with an injected vascular network. The actual vessel diameter (D) was determined using an eyepiece micrometer at microscope magnifications of ×20 objective with ×8 eyepiece and ×10 objective with ×8 eyepiece, taking into account the scale division value (C) using the formula: $D=d \cdot C$, where: D is the actual vessel diameter, d is the measured vessel diameter, C is the calibration coefficient of the ocular micrometer scale. The calibration value (C) of the eyepiece micrometer was determined using a standard microscope grid. According to the specifications, the side length of a small square in the grid is 50 μm. When using a ×10 objective and ×8 eyepiece, the calibration value (C) is 100 μm (0.1 mm), while with a ×20 objective and ×8 eyepiece, it is 50 μm (0.05 mm).

The capillary density was quantified by calculating the number of vessels per unit area, with the unit area corresponding to the field of view of the microscope. The tissue trophic activity index (μm), also referred to as the diffusion radius, was determined by measuring the intervascular distance between two adjacent vessels and dividing the obtained value by two.

Statistical analysis of the obtained data was conducted using the InStat software package, which is specifically designed for the statistical processing of biomedical and epidemiological research data.

The long-term effects of opioid exposure in white rats were modeled through daily administration

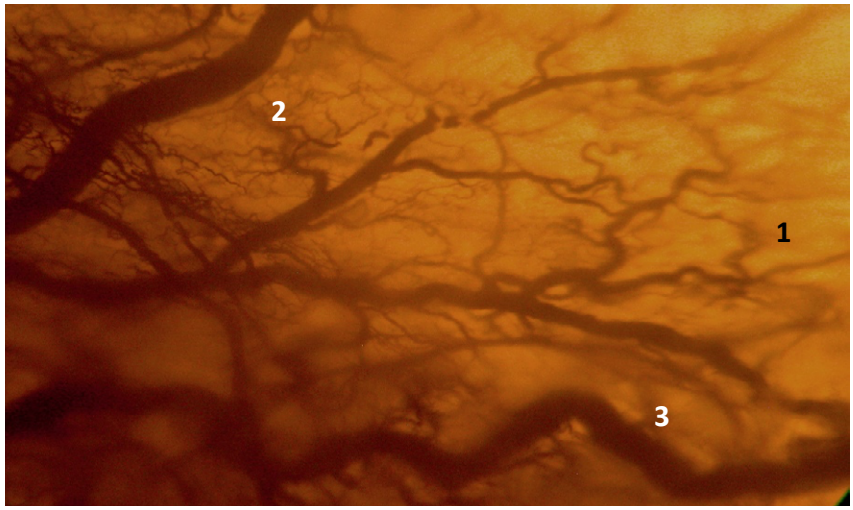

Fig. 1. Subpapillary arterial network and venous plexus of the skin of the white rat's gluteal region after 2 weeks of nalbuphine administration. Microphotograph. Vascular injection. Magnification: objective ×20, eyepiece ×8. 1 – Intrapapillary capillary loop; 2 – Arteriole; 3 – Venule

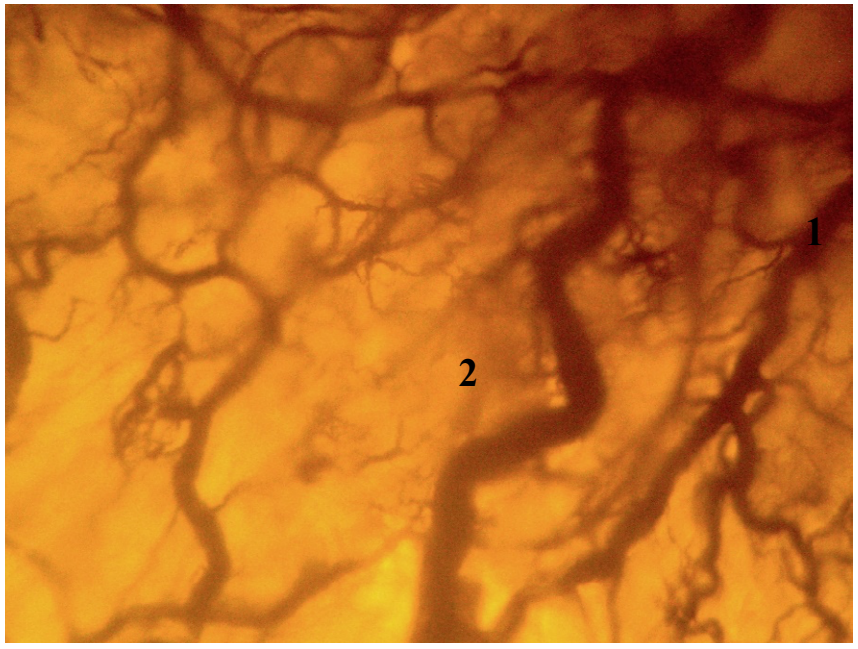

Fig. 2. Dermal arterial network and venous plexus of the skin in the gluteal region of a white rat after two weeks of nalbuphine administration. Micrograph. Vascular injection. Magnification: obj. ×20, ocular ×8. 1 – arteriole; 2 – venule

(once per day at the same time) of the opioid analgesic nalbuphine. Nalbuphine was administered intramuscularly according to the following dosage regimen: week 1 – 8 mg/kg, week 2 – 15 mg/kg, week 3 – 20 mg/kg, week 4 – 25 mg/kg, week 5 – 30 mg/kg, week 6 – 35 mg/kg. The experimental exposure periods lasted two, four, and six weeks from the start of the experiment. This model, which involves a gradual increase in the dose of an accessible opioid analgesic, enables the investigation of its effects on the experimental animals and allows for the assessment of the progressive development of morphological changes in tissues. The proposed method, characterized by weekly dose increments, facilitates the gradual adaptation of opioid receptors in the experimental animals. Age-matched intact animals were used as the control group and received intramuscular injections of a 0.9% sodium chloride solution.

RESULTS

In the skin samples obtained from the gluteal region of the white rat with the injected vascular network, after two weeks of experimental exposure, the following structures were observed, similar to those in the control group: the subpapillary and dermal arterial networks, the subpapillary and dermal venous plexuses, and the subcutaneous venous plexus. The subpapillary arterial network and subpapillary venous plexus are located between the papillary and reticular layers of the skin (Fig. 1).

This is a fine-looped plexus. The capillaries form intrapapillary capillary loops, which have the shape of glomeruli or lace-like networks.

At the boundary between the reticular layer of the skin and the hypodermis lies the dermal vascular plexus. This plexus contains arterioles that form the dermal arterial network and venules that lie deeper, creating the deep dermal venous plexus (Fig. 2). Another vascular plexus is

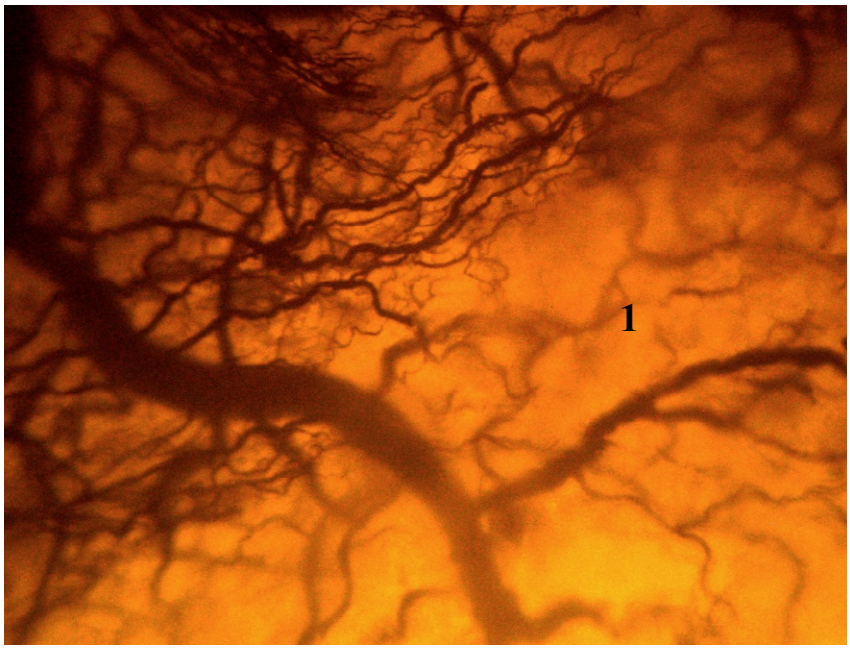

Fig. 3. Subpapillary arterial network and venous plexus of the gluteal skin in a white rat after four weeks of nalbuphine administration. Micrograph. Vascular injection. Magnification: obj. ×20, ocular ×8. 1 – intrapapillary capillary loop

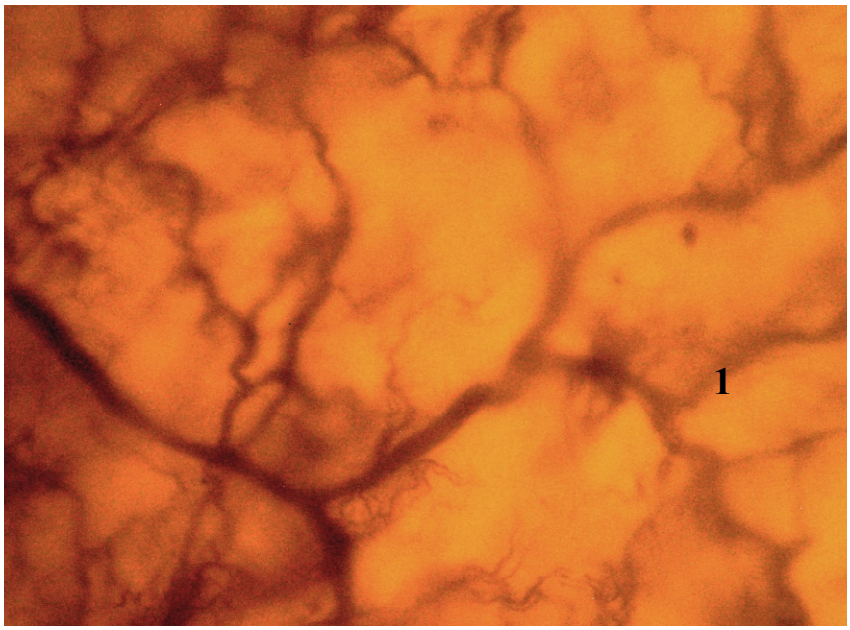

Fig.4. Subcutaneous venous plexus of the gluteal skin in a white rat after six weeks of nalbuphine administration. Micrograph. Vascular injection. Magnification: obj. ×20, ocular ×8. 1 – venule

located in the hypodermis — the subcutaneous plexus. This plexus has a wide-looped structure.

After just two weeks of the experiment, the arteriolar diameter significantly increases. Specifically, the diameter of the arterioles in the subpapillary arterial network reaches 28.62±1.07 μm (control – 22.24±0.73 μm), while in the dermal arterial network, it reaches 49.94±1.74 μm (control – 36.86±1.90 μm). The arterioles appear tortuous. The intrapapillary capillary loops of the skin exhibit slight dilation, with their diameter increasing to 6.20±0.11 μm (control – 5.91±0.26 μm). However, at this stage of the experiment, no significant change in the venular diameter is observed. The diameter of venules in the subpapillary venous plexus is 54.32±0.30 μm (control – 53.97±0.92 μm), while in the subcutaneous venous plexus, it measures 114.93±2.78 μm (control – 112.01±2.25 μm). These changes lead to alterations in other morphometric parameters of the vascular architecture of the white rat's skin under conditions of two-week nalbuphine administration. Notably, the trophic activity index of the skin tissue significantly decreases to 20.36±3.07 μm (control – 33.82±1.38 μm). However, the density of intrapapillary capillary loops in the white rat's skin remains unchanged at this experimental stage.

After four weeks of nalbuphine administration in white rats, the arteriolar lumen exhibits irregularities, characterized by alternating regions of constriction and dilation. Venules undergo expansion, structural deformation, and acquire an irregular morphology,

Table 1. Density of the intrapapillary capillary loop network in the gluteal skin of the white rat

Observation periods	Values	
	M±m (μm)	p
Control	71.000±4.728	p > 0.05
In two weeks after nalbuphine injection	68.000±3.926	p > 0.05
In four weeks after nalbuphine injection	69.200±4.761	p < 0.01
In six weeks after nalbuphine injection	59.000±1.963	p < 0.01

Table 2. Trophic activity index of the gluteal skin tissue in the white rat

Observation periods	Values	
	M±m (μm)	p
1	2	3
Control	33.822±1.376	p > 0.05
In two weeks after nalbuphine injection	20.364±3.066	p < 0.01
In four weeks after nalbuphine injectionIn two weeks after nalbuphine injection	22.472±2.438	p < 0.01
In six weeks after nalbuphine injection	40.410±4.508	p < 0.01

Table 3. Diameter of arterioles in the subpapillary arterial network of the gluteal skin in the white rat

Observation periods	Values	
	M±m (μm)	p
1	2	3
Control	22.240±0.730	p > 0.05
In two weeks after nalbuphine injection	28.616±1.069	p < 0.01
In four weeks after nalbuphine injection	29.540±0.894	p < 0.01
In six weeks after nalbuphine injection	30.120±0.039	p < 0.01

Table 4. Diameter of venules in the subpapillary venous plexus of the gluteal skin in the white rat

Observation periods	Values	
	M±m (μm)	p
1	2	3
Control	53.968±0.919	p > 0.05
In two weeks after nalbuphine injection	54.324±0.298	p < 0.01
In four weeks after nalbuphine injection	59.648±0.832	p < 0.01
In six weeks after nalbuphine injection	62.644±2.080	p < 0.01

with occasional aneurysmal protrusions. Additionally, isolated saccular aneurysms are observed in the microvasculature. These vascular alterations lead to modifications in the spatial organization of the subpapillary and dermal arterial networks, as well as the subpapillary, dermal, and subcutaneous venous plexuses in the gluteal skin of white rats following four weeks of nalbuphine exposure (Fig. 3).

At this stage of the experiment, the subpapillary vascular plexus of the white rat's skin is characterized by the following morphometric parameters: the diameter of the intrapapillary capillary loop is 6.26±0.12 μm, the trophic activity index of the skin tissue is 22.47±2.44 μm, and the diameter of venules in the subpapillary vascular plexus is 59.65±0.83 μm. The diameter of arterioles in the dermal arterial network increases to 55.18±4.18 μm, while the diameter of venules in the dermal venous plexus expands to 142.31±2.46 μm, and in the subcutaneous venous plexus, it reaches 158.57±1.98 μm.

The density of the intrapapillary capillary loop network in the gluteal skin of the white rat is presented in Table 1. A reduction in capillary loop network density to 69.200±4.761 (control – 71.000±4.728) after four weeks of nalbuphine administration indicates a minor disruption in the vascular architecture of the skin at this stage of the experiment.

After six weeks of the experiment, destructive changes in the angiographic pattern of the gluteal skin are observed. The subpapillary arterial network loses its delicate, lace-like appearance. Capillary loops become

dilated and coarse, with some areas showing a loss of capillary connections.

At this stage of the experiment, the diameter of the remaining intrapapillary capillary loops in the gluteal skin of the white rat significantly increases to 6.46±0.19 μm. Additionally, the density of intrapapillary capillary loops decreases markedly, reaching 59.0±2.0.

Changes in the trophic activity index of the skin tissue, as well as the diameters of the arterioles and venules in the subpapillary vascular plexus, are presented in Tables 2–4.

The arterioles of the dermal arterial network appear dilated, tortuous, and irregular in contour. Their diameter increases to 62.46±2.12 μm, while the diameter of venules expands to 158.60±1.99 μm.

Additionally, new arteriovenular anastomoses open, allowing blood to bypass the damaged capillaries and flow directly from the arterioles into the venules. The venules of the subcutaneous venous plexus are dilated and deformed (Fig. 4).

The diameter of venules in the subcutaneous venous plexus of the gluteal skin reaches 162.86±1.94 μm.

Therefore, the statistically significant decrease in the density of the intrapapillary capillary loop network within the subpapillary vascular plexus of the gluteal skin in white rats after four and six weeks of nalbuphine administration, coupled with a significant increase in the trophic activity index of skin tissue, provides compelling evidence of pronounced degenerative alterations in the vascular architecture of the skin resulting from prolonged opioid exposure.

DISCUSSION

Histological analysis of gluteal skin preparations from white rats with injected vascular networks following two weeks of experimentation revealed a clear differentiation of the subpapillary, dermal, and subcutaneous venous plexuses, comparable to that observed in the control group. The scientific literature provides limited data on the structural organization and vascularization of the skin in experimental animals. Existing studies primarily distinguish three vascular plexuses: hypodermal, subdermal, and subpapillary. The present study establishes that two weeks of nalbuphine administration induces a statistically significant increase ($p < 0.05$) in the diameter of arterioles within the subpapillary and dermal arterial networks, accompanied by increased arteriolar tortuosity. These vascular alterations correlate with a significant reduction in the trophic activity index of skin tissue, suggesting an early opioid-induced disruption of cutaneous microcirculation. The vasodilatory effect, specifically the increase in arteriolar diameter observed with nalbuphine administration over varying durations, has been consistently documented by researchers investigating the impact of nalbuphine on the vascular systems of various organs [19]. However, the morphometric parameters presented herein, which are critical for an objective evaluation of skin angioarchitectonics, offer novel contributions to the existing body of knowledge.

The scientific literature suggests that alterations in the skin, associated with various pathological conditions, are primarily accompanied by changes in its microcirculation network [20]. Our findings support these observations. Specifically, after four weeks of nalbuphine administration to white rats, histological examination of skin preparations with injected vascular networks revealed indistinct arteriolar contours and the presence of isolated aneurysmal sacculations within the microvessels. The aforementioned changes result in alterations to the spatial configuration of the subpapillary and dermal vascular networks, as well as the subcutaneous venous plexus in the gluteal skin of white rats following four weeks of nalbuphine administration. After six weeks of nalbuphine exposure, destructive changes are observed in the angiographic pattern of the gluteal skin. Specifically, the subpapillary vascular plexus loses its delicate, lace-like architecture, with capillary loops becoming dilated and coarse, and in certain areas, capillary connections are disrupted. Morphometric analysis of the vascular architecture of the skin under prolonged opioid exposure indicates structural degradation of the vascular network.

CONCLUSIONS

1. The morphological effects of nalbuphine on the white rats' skin become apparent after two weeks of administration, with the first observable changes occurring in the vascular structures of the blood plexuses. Histological analysis of skin specimens with injected vascular networks reveals dilatation of arterioles and capillaries. Specifically, the diameter of arterioles within the subpapillary arterial network significantly increases to 28.62±1.07 μm (control: 22.24±0.73 μm), while the diameter of the subpapillary capillary loops increases to 6.20±0.11 μm (control: 5.91±0.26 μm). The arterioles exhibit tortuosity.
2. After four weeks of experimental exposure, the loops of the vascular plexuses exhibit loss of their previously fine and regular pattern, with the appearance of microaneurysms in arterioles and saccular dilatations in venules. Following six weeks of opioid exposure, significant structural alterations are observed in the

vascular network of the skin. Capillaries are obliterated, and several capillaries are destroyed, a process accompanied by hemorrhages. There is a statistically significant reduction in the density of subpapillary capillary loops, which decreases to 59.0±2.0 (control: 79.60±2.078), while the trophic activity of the tissue increases to 39.490±1.307 μm (control: 27.172±1.143 μm), providing further evidence of the profound destructive changes in the vascular plexuses of the skin.

3. The morphometric analysis of the vascular plexuses in the skin provides clear evidence of the relationship between quantitative and qualitative alterations in the structural organization of the vessels within the microcirculation network in experimental animals subjected to opioid exposure. This analysis enables an objective evaluation of the extent of these changes in correlation with the duration of the experiment.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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RECEIVED: 14.03.2025
ACCEPTED: 07.05.2025