



# Study to elucidate the pharmacological activity of retinalamin in a rat model of ischemic retinopathy

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## Abstract

**Introduction:** Over the past few years, the incidence of retinal ischemic disorders has been increasing, due to a rising prevalence of such socially burdensome diseases as diabetes and hypertension, which ultimately lead to ocular vascular pathology. The identification of new treatment options that would prevent retinal neuron death is a crucial task of modern pharmacology.

**Materials and methods:** The research was carried out on male Wistar rats. Retinopathy was modeled by inducing a 30-min ischemic episode, with a 72-hour period of reperfusion and subsequent administration of Retinalamin and Emoxypine for 10 days. The effectiveness of the drugs was evaluated by electroretinographic, ophthalmoscopic and morphological assessments.

**Results and discussion:** On Day 14 of the experiment, a dose-dependent preservation of the electroretinogram b-wave/a-wave amplitude ratio was observed in the animals treated with Retinalamin depending on a dose ( $1.39 \pm 0.06$ ,  $1.46 \pm 0.03$  and  $1.49 \pm 0.04$  in low (0.214 mg/kg), medium (0.428 mg/kg) and high (0.857 mg/kg) Retinalamin dose groups, respectively). The ophthalmoscopic picture of the fundus oculi also improved following the treatment with Retinalamin (1.42, 1.69 and 1.90 times lower ophthalmoscopic scores compared to placebo-treated animals in low, medium and high dose groups, respectively). The morphologic “coefficient of change” applied to ganglion cell layer was 2.2, 1.7 and 1.6 points in low, medium and high dose Retinalamin groups, respectively. These results are significantly different from both intact and placebo group ( $p < 0.05$ ). Based on the aforementioned experimental findings, we conclude that Retinalamin has a retinoprotective effect and is superior to the drug of comparison (Emoxypine).

**Conclusion:** The greatest neuroprotective effects were shown in the groups receiving Retinalamin. In these groups, the ERG b-wave/a-wave amplitude ratio was preserved, the ophthalmoscopic picture was less pathologic and retinal morphology features were close to those of the intact retina.

## Keywords

ganglion cell layer, inner nuclear layer, retina, Retinalamin.

## Introduction

Over the past few years, the incidence of retinal ischemic disorders has been increasing, due to a rising prevalence of such socially burdensome diseases as diabetes and hypertension, which ultimately lead to ocular vascular pathology. In turn, retinal ischemia causes visual impairment and blindness in people of different age groups (Diereev et al. 2020).

Retinal ischemia occurs when the blood supply to the optic nerve and retina is disrupted due to the occlusion of its vessels, trauma, or increased intraocular pressure (IOP). Different forms of retinal ischemia, such as central retinal artery (CRA) occlusion, central retinal vein (CRV) occlusion, glaucoma, traumatic optic neuropathy and diabetic retinopathy are all common causes of visual impairment and blindness (Hayreh et al. 2004; Mukaida et al. 2004). Retinal neurons, especially retinal ganglion cells (RGC), are highly susceptible to ischemia. The diseases accompanied by retinal ischemia lead to retinal nerve fiber layer (RNFL) thinning, resulting from damage or loss of ganglion cells (Dattilo et al. 2018; Pottabatula et al. 2020).

Acute retinal ischemia may also develop as a complication of chronic ischemic damage, caused by carotid artery stenosis, ophthalmic artery stenosis, diabetic retinopathy, degenerative retinal changes, etc. (Dattilo et al. 2018; Pottabatula et al. 2020).

Traumatic injuries of the eye and head, as well as vasospastic diseases are another cause of ocular ischemic damage. In addition to ischemic neuropathy secondary to blood flow alterations in the CRA and CRV, retinal ischemia can result from hypotension, surgical interventions and certain drugs ingestion. Risk factors predisposing to retinal ischemia include anemia, microembolization, and coagulation disorders (Biousse 2014).

A better understanding of molecular mechanisms that control post-ischemic retinal degeneration is necessary to develop new treatment strategies and to minimize or to reduce vision loss caused by retinal neurons damage (Shabelnikova et al. 2016). Timely intervention can prevent disease progression and improve outcomes (Biousse et al. 2018).

Recently, new theories have evolved suggesting that the modulation of the neuropeptide system can provide a basis for novel pharmacological approaches to treat retinal ischemia. A neuroprotective effect of different peptide substances has been demonstrated in several retinal disease models (Shabelnikova et al. 2016). Neuroprotection is defined as a process that facilitates structural and functional recovery and regeneration of the nervous system and its cells (Neroev and Zaitseva 2015; Yerichev et al. 2017). Multiple neurochemical modulators in the nervous system have neuroprotective effects. Among them, secretory neuropeptides are broadly expressed throughout both the central and peripheral nervous systems. These secretory neuropeptides generally play the role of "second signals", accompanying "classical" neurotransmitters and helping to make fine adjustments of neurotransmission, thereby

controlling the inhibition/excitation balance (Hoyer and Bartfai 2012). By definition, neuropeptides are small protein-like molecules or, in other cases, "normal" proteins, the primary function of which is neurotransmission (Burbach 2010). Some of them were also found important in regulating the cell death/survival in various neural systems (Linden et al. 2005; Catalani et al. 2017; Reglodi et al. 2017). Particularly, neuropeptides together with their receptors are widely expressed in the mammalian retina, where they are involved in multiple processes both during the development and in adult animals (Bagnoli et al. 2003). Among other functions, neuropeptides participate in the visual information processing.

Neuronal damage triggers a cascade of events leading to DNA fragmentation, phagocytosis, apoptosis, autophagy, necrosis, or other forms of cell death (D'Arcy 2019). However, it is not always possible to distinguish between the initial trigger of this cascade and the ultimate mechanism of cell death. Multiple pathways of neuronal death share common steps, and death is often induced by an interaction with neighboring cells, such as glia. Neurons are susceptible to the majority of common cell death mechanisms, including ischemic death, excitotoxicity, calcium overload and axon disruption-induced death, as well as neurodegeneration associated with cell cycle re-entry. Metabolic imbalance, hypo- or hyperglycemia, hypoxia and accumulation of peroxynitrite and reactive oxygen species are also potential cell death factors (Fricke et al. 2018).

The crucial role of neuronal loss in the pathogenesis of many disorders and a complex interaction of overlapping mechanisms involved in neurodegeneration (Fan et al. 2017) make the deeper understanding of the forms and pathways of neuroprotection a fundamental requirement for the development of new strategies to ameliorate nervous system deterioration (Monteiro et al. 2017). Neuropeptide systems are potential targets in the treatment of neurodegenerative diseases of the retina.

Retinalamin has been shown to mitigate destructive changes in the retinal pigment epithelium (RPE) seen in diverse forms of retinal degeneration, to modulate the activity of retinal cells and to increase the efficiency of the functional interaction between the RPE and photoreceptor outer segments in a variety of pathological conditions (Vajda 2002; Osborne et al. 2004; Egorov 2017; Yerichev et al. 2017). In addition, Retinalamin stimulates the fibrinolytic activity of the blood and has an immunomodulatory effect. Due to its high trophic potential, tissue specificity, capacity to target pathogenic mechanisms and safety, this drug is a promising tool for the treatment of retinal diseases.

Given the reasons mentioned above, it is reasonable to suggest that Retinalamin could have a beneficial effect in the treatment of ischemic retinal diseases.

**The aim of this study** was to evaluate the pharmacological activity of peptide drug Retinalamin in a retinopathy model induced by a transient intraocular pressure increase followed by a reperfusion period.

## Materials and methods

### Animals

Throughout the study and animal handling process, research ethical principles were applied in accordance with the European Convention for the Protection of Vertebrates Used for Experimental and Other Scientific Purposes, CETS No. 123. White laboratory outbred rats were obtained from a nursery at the Research Institute of Pharmacology of Living Systems, Russia. The experiment was conducted on 48 male Wistar rats (6 groups, 8 animals in each group). The animals weighing 180–220 g without any signs of disease were selected for the study and underwent a 10-day quarantine before the experiment. All the manipulations on the rats were performed under general anesthesia induced with intraperitoneal administration of *chloral hydrate*. The experiments were approved by the Local Ethics Committee of Belgorod State National Research University, Belgorod, Russia (Protocol No. 12/19-1).

### Design of the experiment

The animals were divided into 6 groups: 1) intact control group; 2) retinopathy model group (placebo group); 3) group treated with 0.214 mg/kg of Retinalamin; 4) group treated with 0.428 mg/kg of Retinalamin; 5) group treated with 0.857 mg/kg of Retinalamin; 6) group treated with  $\approx 0.003$  mg/kg of *Emoxypine*.

The drugs were administered daily at the same time in the morning for 10 days. To avoid bias, all the animals, excepts those from the intact group, received an intramuscular injection and instillation into the conjunctival sac of the corresponding substances according to the experimental design. The placebo group received *saline solution* (solution for injections, sodium chloride 0.9%, JSC PFK Obnovlenie, Russia) as an intramuscular injection and as instillation into the conjunctival sac. The groups treated with Retinalamin (lyophilisate for preparation of solution for intramuscular and parabolbar injections, 5 mg, GEROPHARM LTD, Russia) received an intramuscular injection of Retinalamin in the doses mentioned above and instillation of *saline solution* into the conjunctival sac. The group treated with *Emoxypine* (eye drops, 1%, Moscow Endocrine Plant, Russia) received intramuscular

saline injection and *Emoxypine* instillation into the conjunctival sac. The corresponding doses of each drug and placebo were administered in equal volumes. The route of administration and the doses were selected based on the instructions for use of both drugs, after the human to animal dose conversion.

The retinopathy model was created by transiently increasing the IOP to 110 mmHg (Peresyphkina et al. 2020) by applying mechanical pressure on the anterior chamber of the eye for 30 minutes. Next, a 72-hour reperfusion followed and after that the drugs were administered.

The electroretinography and ophthalmoscopy were carried out on Days 0, 3 and 14. On the last day of the experiment, the eyes were enucleated for a morphological assessment.

### Electroretinography

The electrophysiological study was performed after 30 minutes of dark adaptation using Biopac System, Inc. (USA) hardware and software, as previously described (Peresyphkina et al. 2018a).

### Ophthalmoscopy

Ophthalmoscopy was performed as described elsewhere (Peresyphkina et al. 2018b), using the OI-78M (Volk Optical Inc, Mentor, OH, USA) lense.

To facilitate statistical analysis, the ophthalmoscopic changes observed in the retinopathy model and treatment groups were evaluated using an ophthalmoscopic score (Table 1). The sum of points for each of the parameters was calculated for all the animals, and the group means were obtained.

### Morphological examination

The eyes were enucleated and immersion-fixed in 10% formalin solution. Then the tissues were embedded in paraffin and the sections were prepared as described elsewhere (Shabelnikova et al. 2016). Leica equipment (Germany) was used for histological processing. Prepared microscope slides were scanned using a Mirax Desk system for microscopic examination, morphometric analysis

**Table 1.** Ophthalmoscopic Scoring System

Parameter	Points assigned		
	0	1	2
General appearance of the retina	Pink, uniform	Pale, with almost white ischemic lesions	Pale, thin, with focal retinal hemorrhages
Optic disc	Salmon pink, margins are well defined	Pale, slightly edematous, margins are blurred	Grey, edematous, margins are blurred
Arteries	Straight course, non-tortuous, uniform caliber, artery-to-vein ratio = 2:3	Narrowed, straight course, uniform caliber, occasional arteriovenous nickings	Thread-like, tortuous, irregular caliber, prominent arteriovenous nickings
Veins	Straight course, uniform caliber, vein-to-artery ratio = 3:2	Tortuous, dilated, uniform caliber	Bayoneting, dilation, irregular caliber, microaneurisms and small hemorrhages along the course
Choroid	Clearly visible in periphery / not visible in peripapillary area	Slightly visible in peripapillary area	Clearly visible throughout / in part of peripapillary area
Pigment	No pigment deposition	Isolated deposits of pigment (dark areas)	Multiple pigment granules deposition (dark areas)

and archiving. Panoramic Viewer 1.15.4. software was used for a morphometric and digital image analysis.

A scoring system was developed to objectively evaluate the observed qualitative changes in the retina. The points were assigned in the following manner: no changes in neuronal structures = 0 points, minimal changes = 1 point, moderate changes = 2 points, prominent changes = 3 points. The "coefficient of change" of the retinal morphology was calculated using formula (1):

$$CC = \frac{(1 \times n + 2 \times n + 3 \times n)}{N} \quad (1),$$

where CC is the "coefficient of change"; n is the number of the retina segments with a corresponding (0; 1; 2; 3) degree of change; N is the total number of studied segments.

### Statistical data management

The results were processed using Microsoft Excel 2016 and Statistica 10 (StatSoft, USA). For quantitative variables, the mean and the standard error of the mean were calculated. For between-group comparisons, the Student's t-test for independent groups was used if the distribution of variables was normal; otherwise, the U-test (Mann-Whitney test) was performed. P-values  $\leq 0.05$  were considered statistically significant (Peresyphkina et al. 2020).

## Results and discussion

### ERG

For each group, the b-wave/a-wave amplitude ratio was calculated (Table 2).

After the retinal ischemia was induced, followed by a 72-hour reperfusion period, this ratio decreased by the average of 27% in all the groups with modelled disease, compared to the intact group (statistically significant difference,  $p < 0.05$ ).

On Day 14, the b-wave/a-wave amplitude ratio in the placebo group reached its minimum, declining by 46.9% from the basal value.

In the group treated with 0.214 mg/kg of Retinalamin, the b-wave/a-wave amplitude ratio fell by 33.8% from the basal value. In the groups treated with 0.428 mg/kg and 0.857 mg/kg of Retinalamin, the pooled b-wave/a-wave amplitude ratio showed the least decrease (29%) and was 1.7 times superior to that of the placebo group. In the Emoxypine group, the b-wave/a-wave amplitude ratio on Day 14 fell by 35%, and was significantly different from that of the placebo group ( $p < 0.05$ ).

In all groups with modelled ischemic retinopathy, the b-wave/a-wave amplitude ratio on Day 14 was significantly different from that of the intact group ( $p < 0.05$ ).

### Ophthalmoscopy results

The ophthalmoscopy results are represented in Table 3. The fundus images of animals from each group are shown in Figure 1.

On average, in the animals subjected to retinal ischemia-reperfusion, the ophthalmoscopic score after the 72-h reperfusion period increased six-fold. The animals had a pale retina, an optic nerve disc edema, pallor and blurred disc margins, changes in the caliber and course of the retinal arteries and veins.

On Day 14, the most prominent fundus changes were detected in the placebo group. The mean ophthalmoscopic score was 9.5 points, significantly different from that in the intact group ( $p < 0.05$ ).

In the groups treated with 0.214 mg/kg and 0.428 mg/kg of Retinalamin, the ophthalmoscopic scores were 1.42 and 1.69 times lower, respectively, and in the group treated with 0.857 mg/kg of Retinalamin – 1.9 times lower than that in the placebo group (both significantly different,  $p < 0.05$ ).

In the Emoxypine group, the ophthalmoscopic score showed the smallest, but also significant difference from

**Table 2.** Results of Electrophysiological Study,  $\mu V$  (mean $\pm$ SEM; n=8)

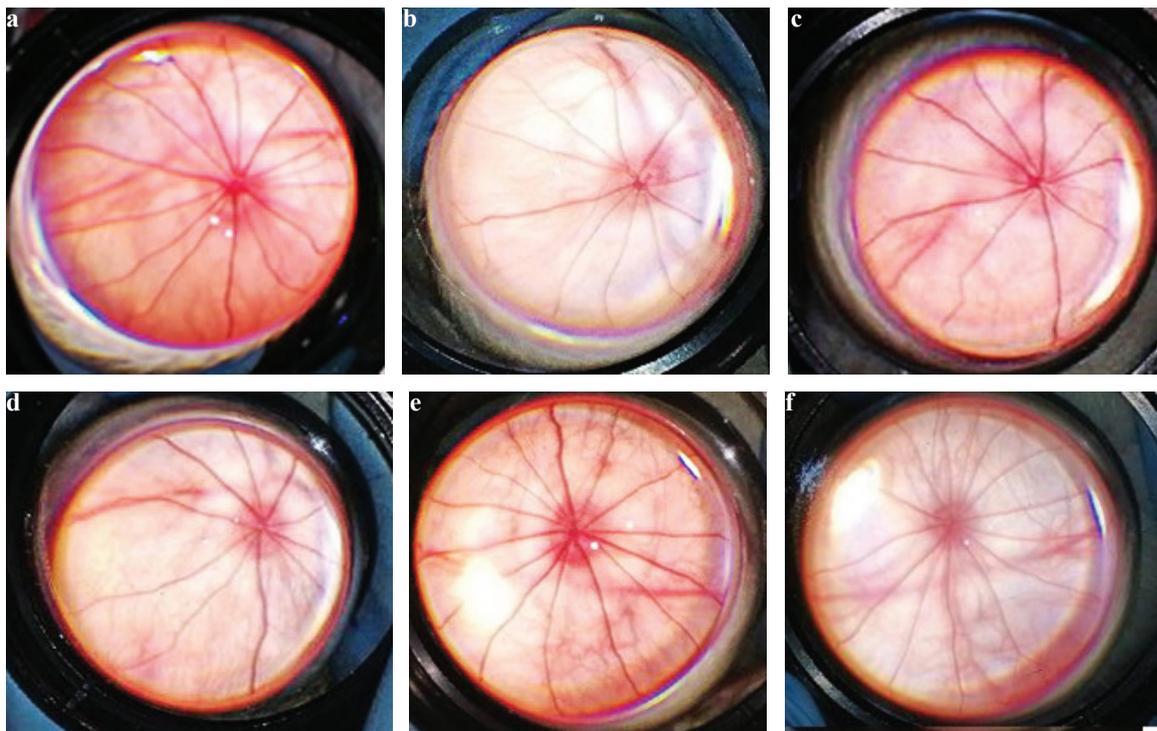
Feature	Intact group	Placebo	Retinalamin			Emoxypine 0.003 mg/kg
			0.214 mg/kg	0.428 mg/kg	0.857 mg/kg	
<b>Day 0</b>						
b-wave/a-wave amplitude ratio	2.04 $\pm$ 0.13	2.09 $\pm$ 0.08	2.1 $\pm$ 0.08	2.06 $\pm$ 0.09	2.1 $\pm$ 0.11	2.05 $\pm$ 0.11
<b>Day 3</b>						
b-wave/a-wave amplitude ratio	2.02 $\pm$ 0.06	1.49 $\pm$ 0.07*	1.56 $\pm$ 0.07*	1.51 $\pm$ 0.06*	1.55 $\pm$ 0.07*	1.55 $\pm$ 0.06*
<b>Day 14</b>						
b-wave/a-wave amplitude ratio	2.05 $\pm$ 0.09	1.11 $\pm$ 0.04*	1.39 $\pm$ 0.06 <sup>y</sup>	1.46 $\pm$ 0.03 <sup>y</sup>	1.49 $\pm$ 0.04 <sup>y</sup>	1.33 $\pm$ 0.04 <sup>y</sup>

Note: \* – significantly different from the intact group ( $p < 0.05$ ); y – significantly different from the placebo group ( $p < 0.05$ ). Student's t-test.

**Table 3.** Ophthalmoscopy Results (Median (QL; QU; n=8))

Feature	Intact group	Placebo	Retinalamin			Emoxypine 0.003 mg/kg
			0.214 mg/kg	0.428 mg/kg	0.857 mg/kg	
<b>Day 0</b>						
points	0.5 (0.0; 1.5)	1.0 (1.0; 1.5)	1.0 (0.5; 1.5)	1.0 (0.0; 1.5)	1.0 (0.5; 2.0)	1.0 (0.0; 1.0)
<b>Day 3</b>						
points	0.5 (0.0; 1.5)	7.0 (5.0; 7.5)*	6.0 (6.0; 7.0)*	6.0 (5.5; 6.0)*	6.5 (5.5; 8.0)*	6.0 (5.5; 7.0)*
<b>Day 14</b>						
points	0.5 (0.0; 1.5)	9.5 (9.0; 10.5) <sup>y</sup>	5.5 (5; 6.5) <sup>y</sup>	5.5 (5; 6.5) <sup>y</sup>	5.5 (4.5; 6.0) <sup>y</sup>	7.0 (7.0; 7.5) <sup>y</sup>

Note: \* – significantly different from the intact group ( $p < 0.05$ ); y – significantly different from the placebo group ( $p < 0.05$ ). Mann-Whitney U-test.



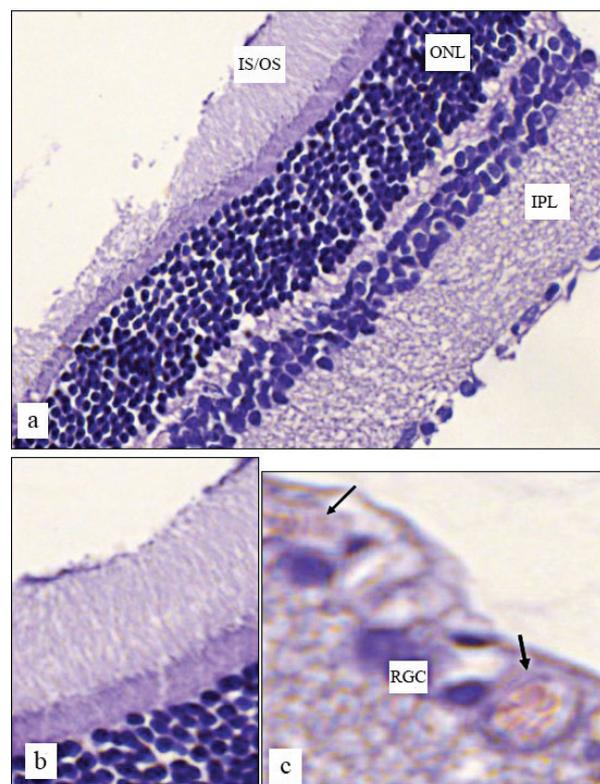
**Figure 1.** Ophthalmoscopic fundus images of the rat eyes on Day 14 of the experiment: (a) animal from the intact control group; (b) animal from the placebo group; (c) animal from the low-dose (0.214 mg/kg) Retinalamin group; (d) animal from the medium-dose (0.428 mg/kg) Retinalamin group; (e) animal from the high-dose (0.857 mg/kg) Retinalamin group; (f) animal from the Emoxypine (0.003 mg/kg) group.

that in the placebo group (1.37 times lower). In all the groups subjected to retinal ischemia-reperfusion, the ophthalmoscopic score on Day 14 was significantly different from that in the intact group ( $p < 0.05$ ).

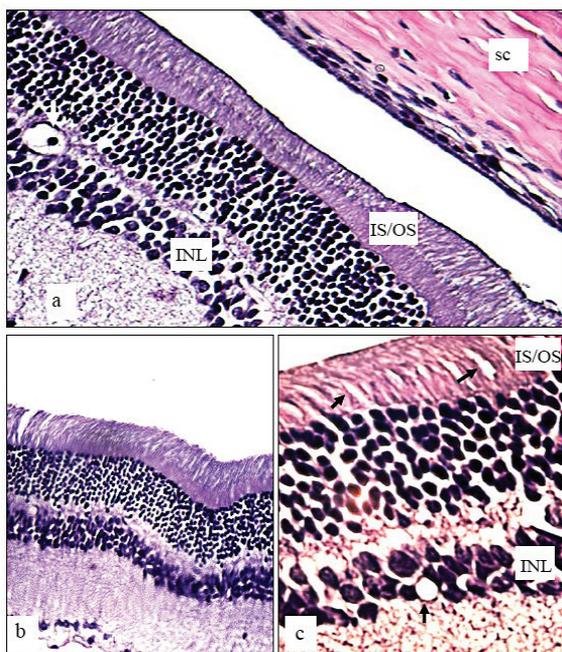
The results of the ophthalmoscopic evaluation suggest that Retinalamin in the studied doses has a beneficial effect on typical ischemic fundus changes, significantly different from both the placebo and intact groups ( $p < 0.05$ ). The greatest effect was detected in the group treated with 0.857 mg/kg of Retinalamin. The animals treated with 0.003 mg/kg of Emoxypine had on average a 1.37 lower ophthalmoscopic score (significantly different from both the placebo and intact groups,  $p < 0.05$ ).

### Histology results

Normal retinal structure (intact animals) is shown in Fig. 2. The retina has an ordered structure with well-differentiated retinal layers formed by neuronal perikaryons (nuclear and ganglion layers) and their processes (reticular layers and the nerve fiber layer). The photoreceptor layer (rods and cones) has a smooth inner and outer border, a relatively homogeneous structure with fine radial striation along the orientation of the photoreceptor dendrites. There are only a few narrow slit-like gaps between them. The outer part of the layer corresponding to the outer segments of the dendrites of the photoreceptor neurons is oxyphilic. The inner part corresponding to their inner segments and the area of the external glial limiting membrane are predominantly basophilic. The outer nuclear layer is formed by densely arranged he-



**Figure 2.** Retinal histology (intact group animal): (a) – general retinal structure, all layers; (b) photoreceptor layer and superficial ONL; (c) GCL (arrows point to venules). Abbreviations: IS/OS – photoreceptor layer (inner and outer segments), ONL – outer nuclear layer, IPL – inner plexiform layer, RGC – retinal ganglion cells layer. H&E stain. Microphotograph. X 200 (a), x 400 (b, c).



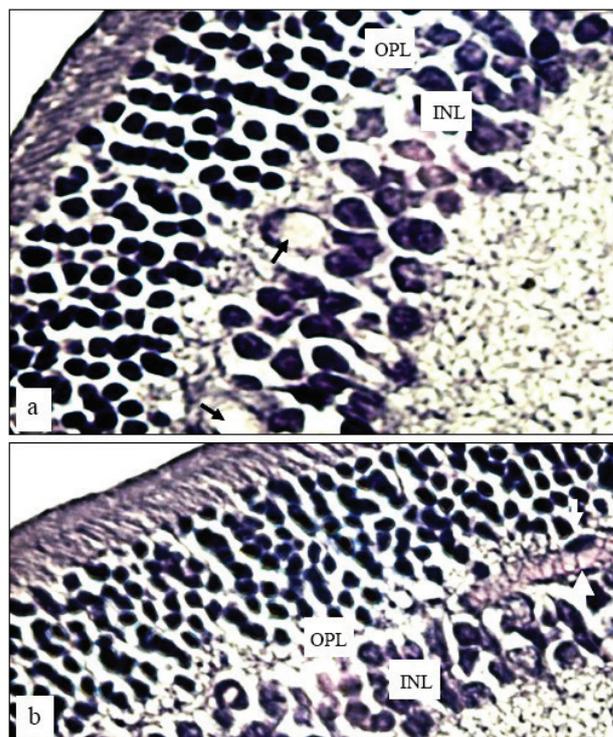
**Figure 3.** General structure of the posterior eye segment (a) and changes in retinal layers (b, c) in the placebo group: a – intact sclera (sc), foci of rarefaction in the photoreceptor layer (IS/OS), perineuronal edema and discrete ballooned neurons (arrow) in the INL; b – area of more preserved retinal structure; c – detailed photoreceptor layer and INL changes (above the arrow: edematous vacuole in the extracellular space). H&E stain. Microphotograph. X 400.

terochromatic nuclei of receptor neurons with a heterochromatin structure.

The plexiform layers have a fine fibrillar structure and, similarly to the GCL, contain microvessels filled with blood to different degrees. The INL contains small fusiform and pyramidal cells with basophilic cytoplasm of variable density and short spiny processes, corresponding more to neuronal elements (bipolar neurons, horizontal and amacrine cells), and more rounded compact cells, corresponding more to radial glial cells. The GCL is formed by multipolar neurons located dispersely or at small distance from each other. These neurons have moderately basophilic homogeneous cytoplasm and nuclei with moderately dense heterochromatin. Basophilic proximal dendritic regions can also be distinguished. A large number of microvessels, mostly postcapillary and collecting venules, moderately filled with blood, can be seen in the GCL.

In the eyes of animals from the placebo group, there were pronounced disruptions of the neural retinal structure, together with other changes indicating accompanying microcirculatory dysfunction. In the photoreceptor layer, there were foci of outer segment fragmentation, appearing as slit-like spaces (Fig. 3 a, c). The outer nuclear layer had foci of neuronal rarefaction.

In the ONL, changes in the venules of the microcirculatory bed expressed as irregular blood filling were predominant, in such a way that both poorly filled venules and vessels with pronounced erythrocytosis and erythrocyte



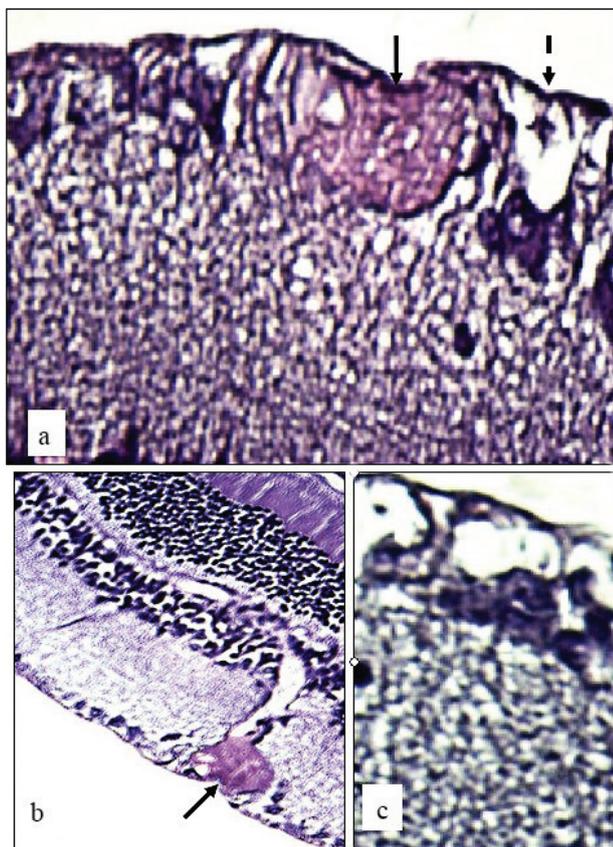
**Figure 4.** Changes in the outer plexiform layer (OPL) and the INL in the placebo group: (a) irregularly filled venules (arrows) in the OPL, dyschromatosis and hyperchromatosis of the INL neurons; (b) venule with erythrocytosis (under the arrow) in the OPL. H&E stain. Microphotograph. X 400.

sludges were present (Fig. 4). The INL neurons showed dyschromatosis and hyperchromatosis of the nuclei and cytoplasm (Fig. 4b).

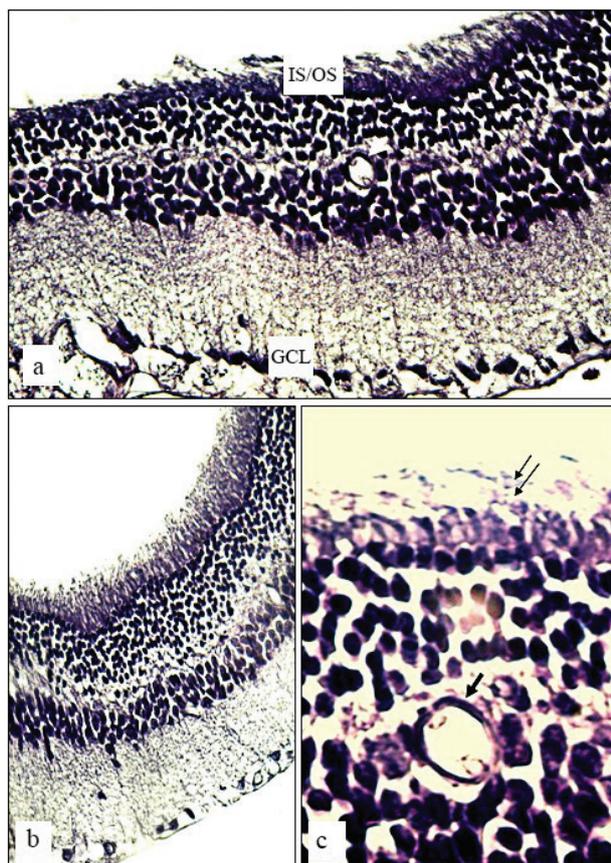
The most prominent changes in both neurons and microvasculature were found in the GCL (Fig. 5). In the microvessels, hemorheological abnormalities were detected mainly in the venules in form of hyperemia and widespread blood stasis (Fig. 5a), with some vessels occluded by fibrin clots (Fig. 5b).

In the context of venous microcirculation abnormalities, severe neuronal damage developed, evident by a diffuse GCL edema, homogenizing changes of ganglion cells (Fig. 5c) and foci of cytolysis. The semi-quantitative coefficient of change (CC) for GCL was 3.0 and was the highest among the groups with modelled retinopathy (morphological examination results by groups are represented in Table 4).

In the animals treated with Retinalamin, the revealed changes were generally stereotypical. Retinas of the animals which had received 0.214 mg/kg of Retinalamine showed an edematous transformation mainly in the photoreceptor layer and GCL, characterized by loose structure of the photoreceptor layer and perineuronal edema in the GCL (Fig. 6). However, the changes of neuronal somas were moderately pronounced, expressed as focal hyperchromatosis. The mean CC was 2.2. The microscopic picture of the ONL was close to normal, indicating preserved viability of photoreceptor neurons.



**Figure 5.** Changes in the GCL in the placebo group: (a) pronounced perineuronal edema, massive erythrocytosis in the vein (under the arrow); (b) blood stasis and fibrin thrombus in the vein (along the arrow); (c) severe homogenizing changes in ganglion neurons, perineuronal edema. H&E stain. Microphotograph. X 400.



**Figure 6.** Histological retinal structure in animal treated with 0.214 mg/kg of Retinalamin: (a) photoreceptor layer rarefaction is evident, apical outer segment parts are fragmented (for details See Fig. c); (b), an empty arteriole in the OPL (for details See Fig. c), perineuronal edema in the GCL. H&E stain. Microphotograph. X 400.

**Table 4.** Morphological Examination Results (Median (QL; QU; n=8))

Feature	Intact group	Placebo	Retinalamin			Emoxypine 0.003 mg/kg
			0.214 mg/kg	0.428 mg/kg	0.857 mg/kg	
Day 14						
CC	0.0 (0.0; 0.2)	3.0 (2.8;3.0) *	2.2 (2.1; 2.4) *y	1.7 (1.5; 1.8) *y	1.6 (1.6; 1.9) *y	2.6 (2.6; 2.8) *y

\* – significantly different from the intact group (p<0.05); y – significantly different from the placebo group (p<0.05). Mann-Whitney U-test.

In the groups of animals treated with 0.428 mg/kg and 0.857 mg/kg of Retinalamin, the retinal structure was similar to that of the intact animals, as evaluated qualitatively (Figs. 7, 8, respectively). The degree of changes in the GCL, both visually and using the CC, was lower in comparison with that in the placebo group, and the group treated with Emoxypine. For the group treated with 0.428 mg/kg of Retinalamin, the CC was 1.65 and for the group treated with 0.857 mg/kg of Retinalamin, the CC was 1.70. In the ONL, the neuronal somas were preserved and photoreceptor neurons had a normal appearance.

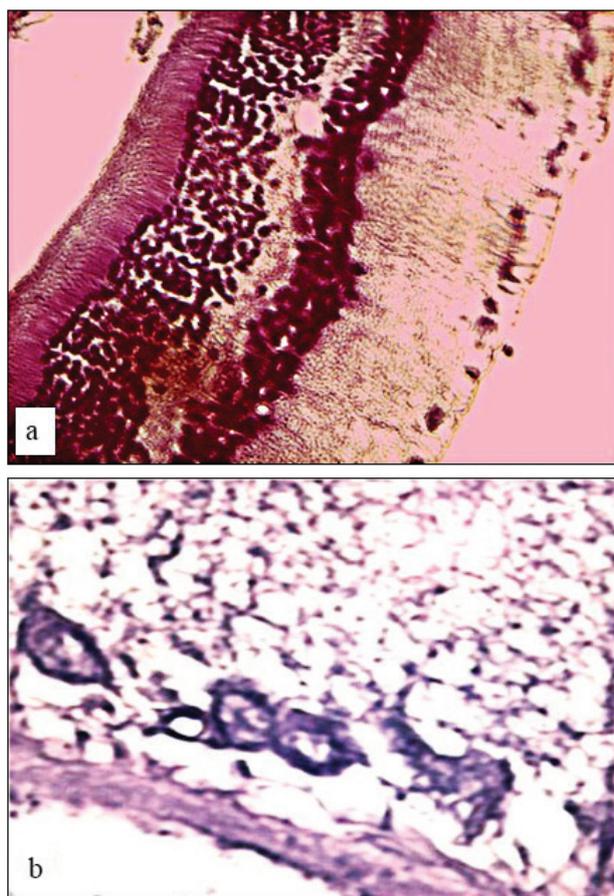
The animals treated with Emoxypine demonstrated less pronounced retinal changes compared to the placebo group (Fig. 9). These changes were mainly characterized by edema and neuronal rarefaction and were most evident in the GCL. The difference between the placebo and

the Emoxypine group was also reflected by the CC of the GCL, which was smaller in the Emoxypine group, equaling 2.63 points.

## Conclusion

Our experimental results show that the 30-minute ischemia followed by reperfusion causes characteristic electrophysiological, ophthalmoscopic and morphological retinal changes.

The greatest neuroprotective effects were shown in the groups receiving Retinalamin. In these groups, the ERG b-wave/a-wave amplitude ratio was preserved, the ophthalmoscopic picture was less pathologic and retinal morphology features were close to those of the intact re-



**Figure 7.** Histological structure of the retina of the animal treated with 0.428 mg/kg of Retinalamin: foci of edema in the GCL, other layers with relatively preserved structure. H&E stain. Microphotograph. X 400.

tina. Retinalamin activity was dose-dependent, starting to be evident at the lowest dose.

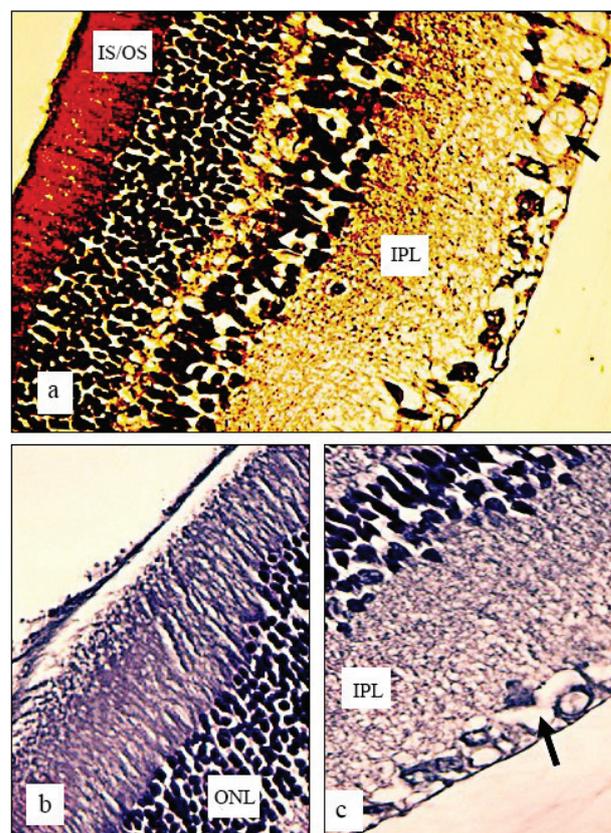
At the same time, the comparison drug **Emoxypine** was inferior in its neuroprotective pharmacological activity to the studied drug. The smaller effect of **Emoxypine** may be explained by its route of administration. The drug concentration reached by instillation into the conjunctival sac is probably low and consequently the drug effect is only modest.

Given the pharmacological activity of Retinalamin in relation to ischemic retinal damage, a detailed study of its mechanism of action in relation to retinal structures is warranted.

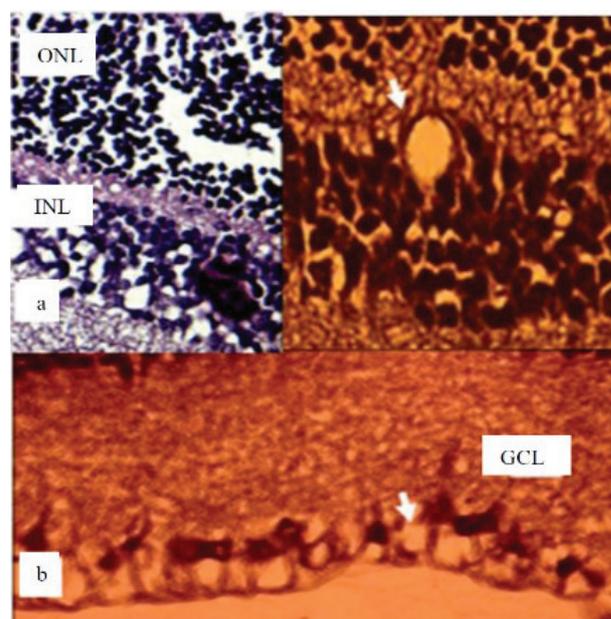
It is possible that due to the peptide structure of the drug it acts via G-protein-bound receptors (GPCRs) (Linden et al. 2005). GPCRs are widely expressed in the retina, which could allow the drug to have an extensive beneficial effect, reducing neuronal cell death (Monteiro et al. 2017).

## Conflict of interests

The authors declare no conflict of interests.



**Figure 8.** Histological structure of the retina of the animal treated with 0.857 mg/kg of Retinalamin: foci of perineuronal edema in the GCL (arrow in Fig. 8c) with partially preserved neurons, structure of other retinal layers similar to that seen in the group treated with 0.428 mg/kg of Retinalamin. H&E stain. Microphotograph. X 400.



**Figure 9.** Histological structure of the retina of animals with retinopathy treated with Emoxypine: the most significant changes are seen in the GCL (arrows). H&E stain. Microphotograph. X 400.

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