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Article

Effects of the Mongolian Medicine Eerdun Wurile on the Expression of Fas and FasL Post Retinal Ischemia-Reperfusion Injury in Rat

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Abstract: The purpose of this study is to examine how the traditional Mongolian medicine Eerdun Wurile affects Fas and FasL proteins following retinal ischemia-reperfusion injury (RIR) in rats. We used healthy adult rats (n=68) dividing them randomly into 5 groups: a control (n=4), a model (n=16), and three Eerdun Wurile treatment groups (n=16 each): high, medium, and low–dose group. The RIR rat model was created using the high intraocular pressure method. Eerdun Wurile was administered via gavage twice daily for a week prior to modeling, while the model group received a medium dose of normal saline. We utilized hematoxylin and eosin (HE) staining to observe retinal morphological changes under an optical microscope. Additionally, we employed immunohistochemical staining to determine how the treatment affected protein expression in the retinas of RIR rats. We observed that 24 hours after RIR, the retinal layers exhibited significant edema, disorganized structure, nuclear atrophy, and a substantial infiltration of inflammatory cells in the ganglion cell layer, accompanied by a reduction in ganglion cell count. As time progressed to 48 and 72 hours post-RIR, the retinal swelling gradually diminished, the retina became thinner, and numerous cells displayed vacuolation and nuclear condensation. Our findings suggest that Eerdun Wurile mitigates retinal cell damage caused by ischemia-reperfusion in rats by suppressing the expression of Fas and FasL proteins in rat retinal cells following RIR induction. Notably, the high-dose Eerdun Wurile group demonstrated significantly greater efficacy compared to the medium- and low-dose groups.

Keywords: Mongolian Medicine Eerdun Wurile; Rats; Retinal Ischemia-Reperfusion Injury; Fas; Fasl

1. Introduction

Retinal ischemia-reperfusion (RIR) represents a widespread pathological mechanism in the field of ophthalmology [1]. This condition is frequently observed in various eye disorders, including glaucoma and central retinal artery occlusion, which can result in damage to the optic nerve and retinal function, potentially leading to vision loss. Numerous strategies have been employed in ophthalmology to prevent the onset of RIR [2]. With the in-depth study of the mechanism of RIR-induced optic nerve damage, it has become evident that simply preventing and treating ischemia or reducing its duration are no longer the sole approaches to managing RIR [3]. To safeguard the patient's remaining visual capabilities, additional factors need to be considered. The study of optic nerve preservation is currently a prominent area of investigation. This research is vital for the treatment and recovery of visual func-

tion in patients with irreversible eye conditions that lead to blindness. In ophthalmology, ischemic retinal damage is a prevalent clinical issue and a leading cause of vision loss. Several key factors play a role in retinal ischemia-reperfusion (RIR), including the infiltration of leukocytes, excessive intracellular calcium, an abundance of free radicals, inflammatory reactions, alterations in nitric oxide (NO) levels and nitric oxide synthase (NOS) activity, as well as genes associated with apoptosis of retinal neurons [4–7].

The primary mechanism of RIR is cellular apoptosis. A crucial apoptotic pathway is mediated by death receptors on the cell membrane, with Fas and Fas ligand (FasL) proteins being central to this process [8]. Fas, also referred to as APO-1/CD95, and FasL, a transmembrane and secreted type II protein, are key members of the tumor necrosis factor receptor family [9]. The binding of Fas to FasL triggers Caspase-3 (cysteine-containing aspartate protease-3), also known as death protein, in the apoptotic pathway, initiating a cascade reaction that results in cell death [10]. Eerdun Wurile, a traditional Mongolian medicine, is frequently used to treat neurological conditions including nerve damage, radiculitis, hypertension, and coronary heart disease. Our earlier research demonstrated that Eerdun Wurile exhibits neuroprotective effects in RIR-affected rats, with its mechanism linked to the reduction of neuronal apoptosis [11]. However, additional research is necessary to elucidate how Eerdun Wurile decreases neuronal apoptosis in RIR rats. In this study, we created an RIR model using elevated intraocular pressure. Eerdun Wurile was orally administered as an intervention to investigate its mechanism for reducing retinal cell apoptosis in RIR rats.

2. Materials and Methods

2.1. Experimental Animals and Grouping

The study utilized 68 healthy adult male Sprague Dawley rats, each weighing 280±20 grams, obtained from Beijing Weitong Lihua Experimental Animal Technology Co., Ltd. (certificate number 170307120118). These rats were housed in the Mongolian Medicine Processing Experimental Center's animal facility at Inner Mongolia Medical University. The housing conditions were specifically pathogen-free, with unrestricted access to water, a standard diet, and a 12-hour light-dark cycle (temperature 18–22 ℃, humidity 40%–70%). The rats were randomly assigned to five groups: control (4 rats), model (16 rats), and three EerdunUriel dosage groups - high (16 rats), medium (16 rats), and low (16 rats). The rat RIR model was created using the high intraocular pressure method, with rats further divided into four subgroups based on post-modeling time points: 6h, 24h, 48h, and 72h. Based on previous research and literature [12], the Eerdun Wurile groups received doses of $0.6g{\cdot}kg^{-1}$, $0.3g{\cdot}kg^{-1}$, and $0.1g{\cdot}kg^{-1}$ for high, medium, and low doses respectively, administered twice daily for 7 days prior to modeling. The model group was administered with medium-dose saline.

2.2. Reagents

The International Mongolian Medicine Hospital of Inner Mongolia Autonomous Region supplied Eerdun Wurile (approval number M14010081). Beijing Life Science Technology Co., Ltd. provided Fas and FasL antibodies, an anti-rabbit two-step kit, and a DAB colorimetric kit. Biyuntian Biotechnology Co., Ltd. was the source of the HE staining kit.

2.3. Establishment of the RIR Model

The RIR model in rats was created by inducing ischemia through elevated intraocular pressure via anterior chamber perfusion [13]. The rats were weighed and sedated with an intraperitoneal injection of chloral hydrate (100 mL/L, 3 mL/kg). Once adequately anesthetized, the animals were secured in a prone position on a platform. The right eye was chosen for the procedure. Pupil dilation was achieved using the compound tropicamide, while oxybuprocaine hydrochloride eye drops provided local anesthesia to the ocular surface. A saline-perfused No. 4 semi-scalp needle was inserted upwards into the rat's anterior chamber, following the temporal corneal scleral limbus level. The needle was secured to the rat's ipsilateral ear edge using adhesive tape to prevent damage to the lens and iris. The infusion bottle was gradually elevated to a height of 150 cm above the rat's experimental eye. This elevation creates an intraocular pressure of 14.7 kPa, surpassing the systemic circulation systolic pressure and consequently obstructing retinal blood flow, thus achieving ischemia. The rapid whitening of the bulbar conjunctiva and iris was accompanied by retinal pallor, observed through a direct ophthalmoscope, indicating complete

obstruction of the central retinal artery's blood flow. Throughout the procedure, chloramphenicol eye drops were periodically administered to maintain corneal moisture and prevent infection. After 60 minutes, the infusion bottle's height was slowly decreased to the rat's eye level, gradually reducing intraocular pressure and restoring retinal blood supply. The infusion system was then shut off, and the anterior chamber perfusion needle was extracted. The iris and conjunctiva swiftly regained their normal coloration, while the fundus retina appeared orange-red, signifying the reopening of blocked vessels and the establishment of reperfusion, thus confirming the successful creation of the RIR model. Post-operation, erythromycin eye ointment was applied to the conjunctival sac, and the rat was returned to its cage upon regaining consciousness.

2.4. Sample Collection

2.4.1. Specimen Collection, Embedding, and Paraffin Section Preparation

Following reperfusion, the right eyes from both the model and Eerdun Wurile groups were extracted at 6, 24, 48, and 72 hours. These samples were then processed through a series of steps: fixation using $40g{\cdot}L^{-1}$ paraformaldehyde, water rinsing, dehydration via gradient ethanol, xylene-based transparentizing, and finally, wax immersion and embedding to create paraffin blocks. Sections were cut parallel to the optic nerve's sagittal axis, with non-continuous slices approximately 4 μm in thickness. These sections were subsequently placed on slides coated with poly-lysine.

2.4.2. Hematoxylin and Eosin (HE) Staining

The tissue sections embedded in paraffin underwent the following steps: first dewaxed and hydrated, then stained with hematoxylin for 5 minutes. After rinsing with double-distilled water, the sections were differentiated using 1% hydrochloric acid in alcohol by volume, followed by another rinse. Eosin was applied to achieve an orange-red color. The sections were then dehydrated using a gradient of alcohol concentrations, made transparent with xylene, and finally sealed using neutral gum. An optical microscope was used to examine the morphological changes in the retinal cells.

2.4.3. Immunohistochemical Staining

Tissue sections embedded in paraffin were rehydrated and subjected to antigen retrieval through heat treatment. To inhibit endogenous peroxidase activity, the samples were incubated at 37°C with 3% H_2O_2 in deionized water. Following blockage with normal goat serum, the sections were treated with rabbit anti-Fas monoclonal antibody (1:100) and rabbit anti-FasL monoclonal antibody (1:100) separately and stored overnight at 4 °C. After warming to 37 °C for 30 minutes, the samples were washed with PBS before applying anti-rabbit secondary antibody kit components and incubating at 37 °C for 60 minutes. Subsequent to PBS rinsing, DAB was utilized for color development, and hematoxylin was employed for counterstaining. The sections underwent dehydration using a gradient of alcohol concentrations, were cleared with xylene, and mounted using neutral gum. The number of Fas and FasL positive cells in the retinal ganglion cell layer and inner nuclear layer was counted under an optical microscope [14].

2.4.4. Measurement of NO, NOS, SOD and MDA in the Serum of RIR Rats

Following various reperfusion intervals (6h, 24h, 48h, and 72h after surgery), the experimental rats were subjected to deep anesthesia, and 3-4 ml of blood was extracted from their intraperitoneal vein. The blood samples underwent centrifugation to isolate the serum. Subsequently, the serum concentrations of NO and NOS were determined using ELISA, adhering to the protocols outlined in the respective assay kits. At the same post-surgical time points (6h, 24h, 48h, and 72h), rats from each experimental group were humanely euthanized via cervical dislocation. Their eyeballs were promptly removed, cleansed with physiological saline to eliminate blood traces, and then homogenized in the same saline solution. The resulting homogenate was centrifuged, and the supernatant was collected. ELISA was employed to measure the activities of SOD and MDA, following the guidelines provided in the corresponding assay kits.

2.5. Statistical Analysis

Statistical analysis was conducted using SPSS26.0 and GraphPad Prism9.0 software. Results were presented as mean ± standard deviation. To examine differences among groups, one-way analysis of variance was employed, with Dunnett's post hoc analysis utilized for further comparisons. Statistical significance was defined as $p< 0.05$.

3. Results and Discussion

3.1. Immunohistochemical Staining

In the control group rats, the retinal layers exhibited well-defined cells with normal morphological characteristics. The neurons were organized in an orderly fashion, with nuclei displaying clear boundaries and spherical or ovoid shapes, as illustrated in **Figure 1**.

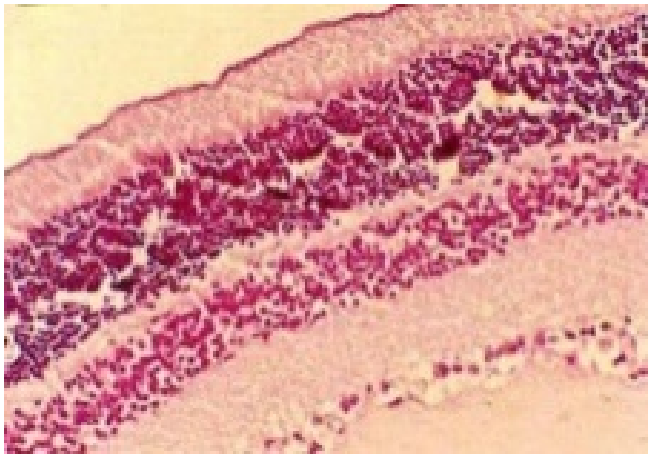

Figure 1. Hematoxylin and eosin staining of retina in a control rat (x200).

Figure 2 illustrates retinal HE staining at 6, 24, 48, and 72 hours following RIR. At 6 hours post-RIR, swelling was observed, cell distribution was sparse, and the ganglion cell layer exhibited a significant increase in inflammatory cells (**Figure 2a–d**). By 24 hours, the retinal layers displayed swelling, disorganization, nuclear atrophy, numerous inflammatory cells in the ganglion cell layer, and a reduction in ganglion cell count. At 48 and 72 hours post-RIR, retinal swelling gradually decreased, the retina became thinner, and many cells developed vacuoles and nuclear condensation. The Eerdun Wurile group showed similar retinal layer changes to the control group. In comparison to the model group, the Eerdun Wurile group demonstrated reduced retinal swelling and molecular layer disorganization, decreased cell death at 72 hours, and a notably thinner retina, as shown in **Figure 2**.

3.2. Protein Expression

We investigated the Fas and FasL protein expression levels in the retina of the rats.

3.2.1. Changes in Fas Protein Expression in the Retina of Rats

Fas was not expressed in the control group. In the model group, Fas protein began to be expressed in the retina 6 hours after RIR, mainly in the layers of nerve fiber and ganglion cells, and parts of the inner plexiform layer; 24 hours after RIR, the positive expression of retinal Fas protein reached a peak, and brown positively stained cells were distributed in the retinal ganglion cell layer, inner plexiform layer, inner nuclear layer, and nerve fiber layer; the expression decreased 48 hours after RIR, and a small amount was expressed after 72 hours. At 6 hours, 24 hours, 48 hours, and 72 hours after RIR, the number of FasL protein expressions in each group was less than that in the model group at each time point. Compared with the model group, the high-, medium-, and low-dose groups of Eerdun Wurile at 6 hours after RIR, the high-dose group of Eerdun Wurile at 24 hours after RIR, the high- and medium-dose groups of Eerdun Wurile at 48 hours after RIR, and the high-, medium-, and low-dose groups of Eerdun Wurile at 72 hours after RIR all showed significant changes.

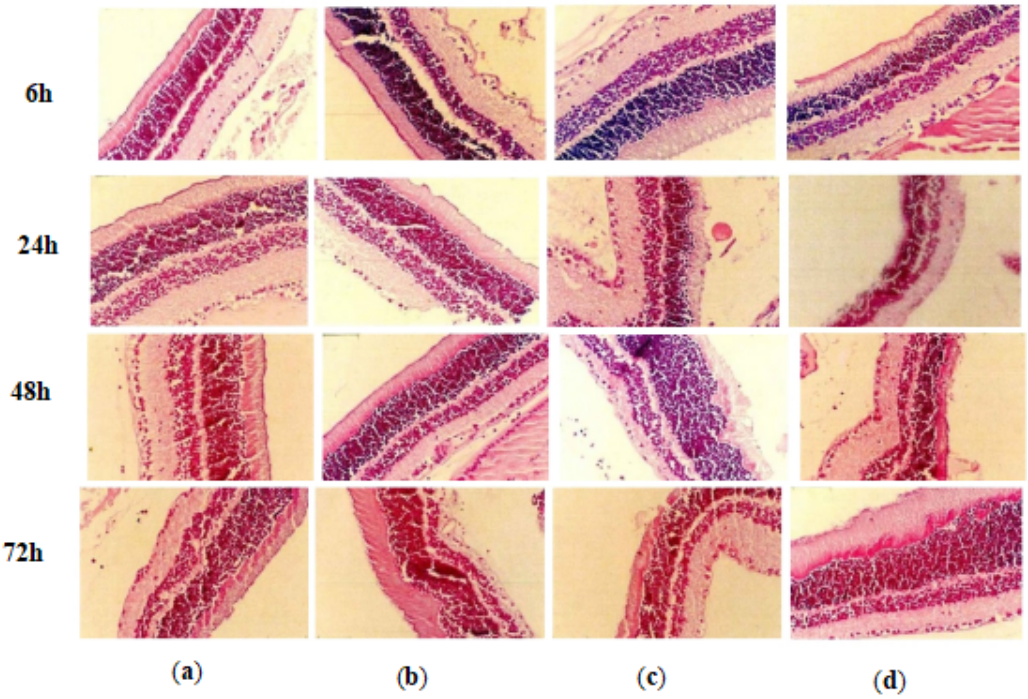

Figure 2. Hematoxylin and eosin staining of retina at 6, 24, 48 and 72 hours after modeling (×200). (**a**) model, (**b**) low- (**c**) medium-, and (**d**) high-dose Eerdun Wurile group. Abbreviations: h, hours.

Fas level was significantly different between the high- and the medium-dose group 24 hours after RIR (p < 0.01), and between the high- and the medium-dose group 48 hours after RIR (p < 0.01). Furthermore, the difference between the high-dose and the medium-dose group 24 hours after RIR (p < 0.05) was investigated and the result was consistent with the levels between the high-dose and the low-dose group at 48 hours after RIR (p < 0.01) shown in **Table 1** and **Figure 3**.

Table 1. Number of cells expressing Fas in each group at 6, 24, 48 and 72 hours post RIR.

Group	n	Fas (cell number/general classifier·mm^{-2})			
		6h	24h	48h	72h
Model	4	194.25±3.304	984.0±46.783	637.25±8.421	98.0±5.598
Low dose	4	177.25±303.4**	967.0±46.783	620.25±8.421$^{▲▲}$	81.0±5.598**
Medium dose	4	175.25±303.4**	894.25±51.99*	587.75±10.34**^{ΔΔ}	77.50±5.066**
High dose	4	170.25±3.304**^{Δ}	795.0±56.515**$^{ΔΔ▲}$	548.25±18.733**$^{ΔΔ▲▲}$	72.0±12.138**

Asterisk (*) expresses statistical significance compared with the model group * p < 0.05 and ** p<0.01 open triangle (^{Δ}) shows the difference compared with the low-dose group ^{Δ} p < 0.05, and ^{ΔΔ} p < 0.01 whilst filled triangle expresses group differences compared with the two groups $^{▲}$ p < 0.05, and $^{▲▲}$ p < 0.01.

3.2.2. Changes in FasL Protein Expression in the Retina of Rats

The expression level of FasL was low in the whole layer of retina of the rats in the control group; whilst in the model group, FasL protein began to be expressed at 6 hours post RIR, mainly in the nerve fiber layer and ganglion cell layer areas; 24 hours post RIR, FasL protein expression reached the peak, dark brown cell membranes and cytoplasm-stained cells can be seen distributed in the retinal ganglion cell layer, inner plexiform layer, inner nuclear layer and nerve fiber layer; 48h and 72h post RIR, the positive expression of FasL protein gradually decreased. At 6h, 24h, 48h, and 72h post RIR, the number of cells expressing FasL protein in each group of Eerdun Wurile was fewer than the number of positive cells at each time point in the RIR group. The number of cells expressing FasL in the high-, medium- and low-dose groups at 6h, 24h, and 48h post-RIR but only the high- and low-dose groups at 72h after RIR was significantly different comparing with that in model group (p < 0.01). The statistical significance still remained in the Eerdun Wurile medium-dose group at 72 hours post RIR compared with that of the rats in the

Eerdun Wurile low-dose group at 24h and 48h post RIR ($p < 0.05$). Furthermore, the number of cells expressing the protein between the high-dose group and the medium-dose group ($p<0.01$) was unlikely at 48 hours after RIR shown in **Table 2**, and **Figure 3**.

Table 2. Number of cells expressing FasL in each group at 6, 12, 24 and 48 hours. post RIR.

Group	n	FasL (cell number/ general classifier·mm^{-2})			
		6h	24h	48h	72h
Model	4	83.75±7.089	949.75±16.194	798.0±16.951	323.75±16.07
Low dose	4	55.75±7.089**	921.75±16.194**	770.0±16.951**	295.75±16.07**
Medium dose	4	62.0±5.354**	904.5±9.883**	749.25±8.139**	301.75±10.966*
High dose	4	55.25±8.539**	887.75±4.787**$^{\Delta\Delta}$	708.0±4.32**$^{\Delta\Delta}$▲▲	290.0±6.683**

Asterisk (*) expresses differences were statistically significant comparing with that of the model group * $p < 0.05$ and ** $p < 0.01$ open triangle ($^{\Delta}$) shows the significant difference compared with the low-dose group $^{\Delta}$ $p < 0.05$, and $^{\Delta\Delta}$ $p < 0.01$ whilst filled triangle expresses group differences compared with the two groups ▲ $p < 0.05$, and ▲▲ $p < 0.01$.

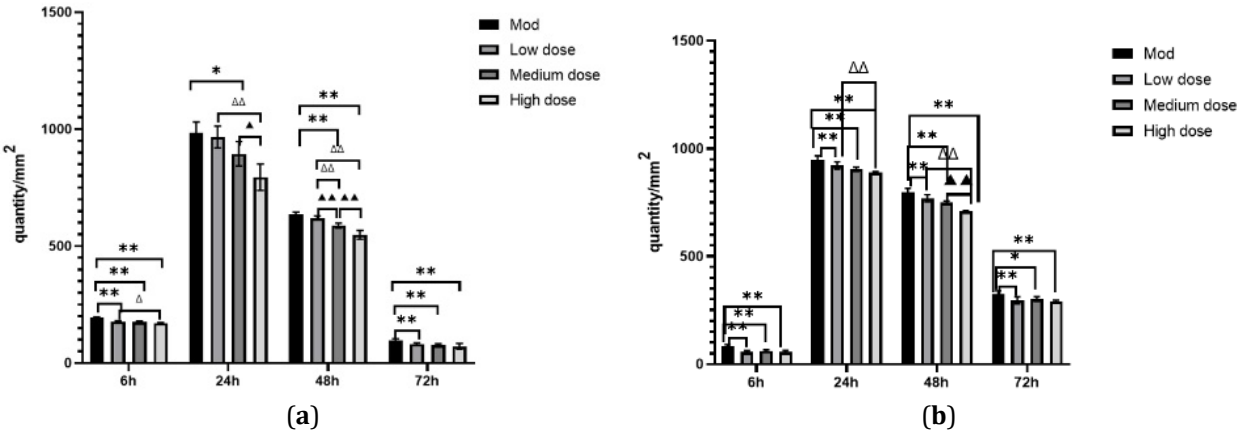

Figure 3. Comparison of the expression levels of (**a**) Fas, and (**b**) FasL in each group at 6, 24, 48, and 72 hours post RIR. Abbreviations: h, hours; mod, model.

3.3. Effects on NO, NOS, SOD and MDA Levels in the Serum of RIR Rats

Table 3 demonstrates that serum levels of NO, NOS, SOD and MDA and NO were notably elevated in the model group compared to the control group ($p < 0.01$). The high-dose group exhibited significant differences than that in the model group at 24, 48, and 72 hours ($p < 0.01$). Furthermore, the low- and medium-dose groups showed statistically significant differences at 48 and 72 hours ($p < 0.05$). No substantial variations were noted in the remaining groups. Serum NOS levels in the model group were markedly higher than those in the control group ($p < 0.01$). The high-dose group displayed statistically significant differences from the model group at 48 and 72 hours ($p < 0.05$). The other groups did not show any differences which were significant.

4. Discussion

Retinal ischemia-reperfusion often leads to retinal cell apoptosis. However, if prompt and effective treatment is administered, RIR can be reversed, the extent of retinal cell apoptosis can be diminished, and compromised retinal cells can be safeguarded [14]. We previously found that the Mongolian medicine Eerdun Wurile has a neuroprotective effect on retinal nerve cells in rats with RIR, and its mechanism was related to reducing the apoptosis of nerve cells in RIR rats [11]. In our current investigation, we employed the intraocular pressure technique to create a rat model of RIR. Our findings demonstrated that following RIR, the rats' retinas became thinner and the quantity of retinal ganglion cells decreased. The results aligned with previous studies reported in the literature [15], confirming the successful establishment of the model. However, in the examination of the Mongolian medicine Eerdun Wurile, while the retinal thickness in each Rile group was reduced, it remained greater than that observed

Table 3. The effect of Eerdun-Wurile on NO, NOS, SOD and MDA (u/mgprot) in serum of RIR rats.

Groups	Parameters	N	Ischemia-Reperfusion Time (h) 4h	24h	48h	72h
Normal	NO	4	1.32±0.44	1.11±0.72	1.07±0.89	1.14±0.48
	NOS	4	0.18±0.03	0.19±0.06	0.20±0.04	0.19±0.06
	SOD	4	111.69±17.39	108.41±18.27	109.44±18.92	112.82±16.52
	MDA	4	2.49±0.57	2.50±0.63	2.38±0.54	2.48±0.74
Model	NO	4	2.47±0.91▲▲	3.03±0.68▲▲	3.12±10.89▲▲	3.24±1.85▲▲
	NOS	4	0.33±0.08▲▲	0.30±0.08▲▲	0.28±0.05▲▲	0.33±0.14▲▲
	SOD	4	78.86±16.63▲▲	80.09±19.45▲▲	79.82±19.49▲▲	77.81±17.81▲▲
	MDA	4	3.43±0.75▲	3.30±0.46▲	3.29±0.48▲	3.46±0.74▲
Low-Dose	NO	4	1.76±0.61	2.23±0.86	2.20±0.84*	1.70±0.80*
	NOS	4	0.30±0.06	0.29±0.07	0.25±0.10	0.30±0.06
	SOD	4	85.06±21.80	85.65±20.86	84.54±20.00	95.33±24.09
	MDA	4	2.79±0.44	2.89±0.52	2.86±0.52	3.02±0.47
Medium-Dose	NO	4	1.87±1.39	2.37±1.03	1.37±0.73*	1.30±0.30*
	NOS	4	0.25±0.06	0.25±0.11	0.21±0.09	0.21±0.10
	SOD	4	90.79±18.66	91.83±22.53	91.14±21.82	97.93±20.13*
	MDA	4	2.66±0.57	2.64±0.59*	2.57±0.67*	2.89±0.53*
High-Dose	NO	4	1.67±1.60	1.67±0.80**	1.33±0.43**	1.21±0.21**
	NOS	4	0.27±0.10	0.25±0.10	0.21±0.08*	0.21±0.07*
	SOD	4	98.86±19.88	99.27±17.44	100.88±18.86*	101.03±21.96*
	MDA	4	2.60±0.46*	2.57±0.63*	2.44±0.67**	2.50±0.43**

Note: Compared with the control group: ▲▲ $p < 0.01$; compared with the model group: ** $p < 0.01$, * $p < 0.05$.

in the RIR group. Additionally, the retinal ganglion cell count was notably higher compared to the RIR group. These observations suggest that EerdunWurile, a Mongolian medicine, exhibits neuroprotective properties against RIR. The mechanism behind this effect may be linked to Eerdun Wurile's ability to decrease retinal cell apoptosis [16]. Although the exact process by which Eerdun Wurile reduces RIR apoptosis remains unknown, this study aims to conduct a thorough investigation into this phenomenon. One of the most crucial pathways for retinal cell apoptosis is the Fas/FasL-mediated death receptor pathway [11]. Fas, a type I cell surface glycoprotein, can initiate apoptotic signals when it binds to its natural ligand, FasL [17]. FasL, which serves as a ligand for Fas, is produced by activated T cells and can be expressed in ocular tissues to trigger apoptosis in infiltrating inflammatory cells or inhibit neovascularization. Besides its pro-apoptotic function, FasL can also stimulate the production of pro-inflammatory cytokines [18]. The binding of Fas to FasL triggers Caspase-3, leading to cellular apoptosis [19]. This research revealed that the control group's retina contained only a small number of Fas and FasL-positive cells. In contrast, 6 hours following retinal ischemia-reperfusion (RIR), the model group exhibited an increased presence of Fas and FasL-positive cells, with their numbers continuing to rise. The concentration of these cells peaked 24 hours post-RIR, remained elevated at 48 hours, and then decreased significantly by 72 hours after RIR. Eerdun Wurile groups exhibited detectable Fas and FasL cells 6 hours post-RIR. These cell counts peaked at 24 hours, followed by a gradual decline. Throughout the observation period, Eerdun Wurile groups consistently showed significantly lower Fas and FasL cell numbers compared to the model group, indicating Eerdun Wurile's neuroprotective effect through Fas and FasL protein expression inhibition. This suggests that Eerdun Wurile may reduce retinal cell apoptosis in RIR rats by suppressing Fas and FasL protein expression. The high-dose Eerdun Wurile group demonstrated markedly fewer Fas and FasL expressing cells than the medium and low-dose groups at all time points, implying superior efficacy of the high-dose treatment compared to lower dosages.

RIR is a prevalent eye disorder that significantly impairs patients' visual acuity and optic nerve functionality. Consequently, there is substantial clinical value in actively studying the mechanisms underlying RIR and identifying effective treatments and medications. While the precise factors causing RIR damage remain uncertain, current studies indicate that it is mainly linked to the infiltration of leukocytes, excessive intracellular calcium, overproduction of free radicals, inflammatory processes, alterations in NO levels and NOS activity, and genes associated with

retinal neuron apoptosis [4–7].

To create a model of retinal ischemia-reperfusion (RIR) injury, we employed a technique involving the insertion of a saline-filled perfusion needle into the rat eye's anterior chamber. The intraocular pressure was elevated by manipulating the perfusion solution's height, inducing retinal ischemia. Subsequently, the height was reduced to normalize intraocular pressure, allowing blood flow to resume, thus mimicking the conditions of RIR injury.

Eerdun Wurile, also known as Zhenbao Pill or Pearl Pill, originates from the Mongolian medical classic Fanghai. It is composed of 29 authentic Mongolian medicinal ingredients [20], including red sandalwood, prepared pearl, nutmeg, bezoar, rhino horn, gardenia, licorice, carpetweed, safflower, clove, cardamom, long pepper, myrobalan, musk, agarwood, cinnamon, and bamboo shavings, among others. EerdunWurile, a prominent remedy in Mongolian medical practice, is frequently prescribed for a range of neurological ailments, including stroke, neuropathy, peripheral neuritis, optic nerve damage, and functional neurosis. This traditional medicine also plays a significant role in addressing cardiovascular disorders such as myocardial infarction, hyperlipidemia, and coronary [21–23]. It is also widely used for difficult-to-treat and unknown conditions and in recent years, research on Eerdun Wurile has deepened, focusing on conditions such as ischemic stroke, atherosclerosis, cerebral ischemia-reperfusion injury, retinal ischemia-reperfusion injury (RIR), diabetic peripheral neuritis, and spinal cord injury [24, 25]. Its main pharmacological actions include scavenging free radicals, improving blood circulation, enhancing capillary permeability, reducing platelet aggregation, preventing thrombosis formation, and inhibiting neuronal apoptosis [26–28]. This research examines how Eerdun Wurile impacts SOD and MDA concentrations in RIR rat retinal tissues, along with serum NO and NOS levels. Reactive oxygen species (ROS) are crucial in RIR-induced damage. Typically, ROS causes damage through four primary mechanisms: (1) damaging nucleic acid sequences, (2) disrupting polypeptide chains, (3) triggering lipid peroxidation, and (4) interfering with energy processes. Retinal ganglion cells (RGCs), abundant in unsaturated fatty acids, are especially susceptible to attacks by reactive oxygen species (ROS). Additionally, ROS stimulates the discharge of excitotoxins, and their combined action induces cell toxicity, resulting in RGC death through apoptosis. The protection of retinal ganglion cells can be achieved through efficient ROS elimination in the retina. A primary mechanism of retinal injury is lipid peroxidation, which occurs when ROS assaults unsaturated fatty acids. In the retina, the membrane discs of photoreceptor cells' outer segments contain high levels of polyunsaturated fatty acids, making them particularly prone to oxidative damage. Malondialdehyde (MDA), the end product of lipid peroxidation, functions as an indicator of the lipid peroxidation level and indirectly measures the extent of tissue damage caused by reactive oxygen species (ROS). Superoxide dismutase (SOD), a crucial ROS scavenger, indicates the organism's capacity to neutralize ROS [29]. In the RIR rat model, we used the ELISA method to measure SOD and MDA levels in retinal tissues. The results showed that, after retinal ischemia-reperfusion, the SOD levels group were significantly lower, but MDA levels were significantly higher in the model than those in the control group. Compared to the model group, the SOD levels were significantly increased but MDA levels decreased in the high-dose group at 48 hours and in the medium- and high-dose groups at 72 hours. Further, there were no differences in the other groups. Nitric oxide (NO) is a physiologically active compound that serves multiple purposes, including the relaxation of smooth muscle in blood vessels, vasodilation, and the prevention of platelet clumping [30]. Nitric oxide (NO), a biologically active molecule, is produced naturally in the body through the conversion of L-arginine by the enzyme nitric oxide synthase (NOS). This endogenous NO plays a significant role in the underlying mechanisms of various medical conditions [31]. In the RIR rat model, we also measured serum levels of NO and NOS using the ELISA method. The results showed that, after retinal ischemia-reperfusion, NO and NOS levels in the model group were significantly higher than those in the control group. We also observed trends as follows: NO levels began to significantly decrease at 24 hours post-reperfusion and peaked at 72 hours, while NOS levels started to decrease significantly at 48 hours and peaked at 72 hours. The high-dose group showed better results compared to the medium-dose group, which outperformed the low-dose group. These findings suggest that Eerdun Wurile can enhance SOD activity and reduce MDA, NO, and NOS levels in RIR injuries. The findings suggest that Eerdun Wurile demonstrates a protective effect against RIR-induced damage and shows promise for clinical applications.

5. Conclusions

Our findings suggest that Eerdun Wurile mitigates ischemia-reperfusion-induced damage to rat retinal cells by suppressing Fas and FasL protein expression. The high-dose Eerdun Wurile group demonstrated notably superior

effects compared to the medium- and low-dose groups.

Author Contributions

Conceptualization, C.L., M.B., D.D., and Y.C.; methodology, C.L., M.B., D.D., Y.C.; software, C.L. and Y.C.; validation, C.L., M.B., D.D., and Y.C.; formal analysis, C.L., M.B., D.D., M.S., T.B. and Y.C.; investigation, C.L. M.B., D.D., and Y.C.; resources, M.B., D.D. and Y.C.; data curation, C.L., M.S. and T.B.; writing—original draft preparation, C.L., M.S. and T.B.; writing—review and editing, C.L., M.B., D.D., M.S. T.B. and Y.C.; visualization, C.L., M.S., and T.B.; supervision, D.D. and Y.C.; project administration, C.L., D.D., and Y.C.. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

The study was conducted in accordance with the Declaration of Helsinki, and approved by the The Inner Mongolia Medical University Ethics Review Committee (protocol code: YKD202404158 on April 15th, 2024).

Informed Consent Statement

Not applicable.

Data Availability Statement

The data that support the findings of the study are available on request from the corresponding author with reasonable request.

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Conflict of Interest

The authors declare no conflict of interest.

Conflicts of Interests

The authors declare that they have no competing interests.

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