THE PROTECTIVE EFFECT OF PUERARIN ON RETINA OF DIABETIC RATS AND INHIBITION OF NF - K B EXPRESSION

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ABSTRACT

Objective: *To investigate the protective effect of Puerarin on the retina of diabetic rats and the inhibition of NF - ϰ B expression.*

Method*: The diabetic retinopathy model was established in rats. Thirty rats were randomly divided into the puerarin group (10 rats), the model group (10 rats), and the normal group (10 rats). Model group: the same amount of saline was injected once a day for 20 days. Normal group: normal feeding. Puerarin group: rats were intraperitoneally injected with Puerarin 10 mg / kg once a day for 20 consecutive days. The general condition, body weight, blood glucose, protein expression and other related indicators were observed and analyzed. Finally, the data were statistically analyzed. In general, there was no significant difference in the initial body weight among the three groups.*

Results*: The body weight of the normal group increased faster than that on the third day after injection (P < 0.001), and the difference gradually increased. There was no significant difference in blood glucose among the three groups, but it was statistically significant compared with the normal group (P < 0.001). Compared with the model group, the expression of PKC protein in the retina of Puerarin group was significantly lower than that of the model group (P < 0.01). In the comparison of curative effect, the recovery, obvious effect, and effective combination were combined. The remission effect of Puerarin group was better than that of the model group.*

Conclusion*: Puerarin can improve vision and hemorheology. Puerarin can protect the diabetic retina by inhibiting PKC activation and NF - ϰ B expression and reduce the expression of VEGF.*

Keywords: *Diabetes Mellitus, Puerarin Research, Retina Research, Nf - ϰ B Expression.*

DOI: 10.19193/0393-6384_2022_3_304

Received *March 15, 2021;* ***Accepted*** *January 20, 2022*

Introduction

It is one of the main retinal vascular diseases which seriously damages visual function, and is also one of the important causes of diabetic blindness [(1-2)]. There are more and more pathological changes such as changes in capillary cell structure and permeability, microcirculation, capillary microaneurysms, and occluded area dysfunction[(3)].

In recent years, the research on clinical treatment, prognosis, histopathology, etiology and pathogenesis of DR in China has gradually deepened[(4-5)]. However, in the treatment of traditional Chinese medicine, the pathogenesis of DR has not been clear, and the treatment has not made a breakthrough[(6)]. Diabetic retinopathy brings great physical and mental pain to patients, seriously affects the quality of human life, and causes heavy economic burden and psychological pressure to the country, society and patients' families[(7-9)]. At present, the principle of modern medical treatment of diabetic retinopathy is: on the basis of diabetes treatment, regular observation of nonproliferative fundus; pre proliferative selective laser photocoagulation; proliferative retinal photocoagulation or vitrectomy[(10-12)]. The long-term side effects of the

patients are too high to accept[13-15]. Chinese medicine has a certain effect on the treatment of diabetic retinopathy[16].

Since ancient times, the treatment of diabetic retinopathy has had a similar record[17]. Some traditional Chinese medicine prescriptions have been used for thousands of years and accumulated rich clinical experience. Traditional Chinese medicine has its unique advantages in the prevention and treatment of diabetic retinopathy, its toxicity and side effects are very small, it is not easy to produce drug resistance, and can improve the ability of the human body to resist disease by improving the internal environment of the human body[18-20]. Compared with western medicine, traditional Chinese medicine has its unique characteristics in the prevention and treatment of diabetic retinopathy, with the characteristics of overall regulation, syndrome differentiation and treatment, and advantages of safety and fewer side effects[21,22]. The early pathological changes of diabetic retinopathy (DR) include the loss of cells around the nipple, the formation of microangioma, the thickening of the capillary basement membrane, the destruction of the retinal blood barrier, bleeding, exudation, and retinal edema[23-25]. Neovascularization, abnormal angiogenesis, and fibroplasia were found in the late stage[26,27]. At present, the understanding of its pathological characteristics mainly focuses on the changes in retinal capillary function and morphology. Functional abnormalities can develop into morphological abnormalities, and morphological abnormalities can aggravate their functional abnormalities, and the two influence each other[28].

In the early stage of DR, there are no conscious symptoms in the eyes, so patients often cannot determine the time of occurrence of eye diseases. The most common complaints were flash sensation and loss of vision. In nonproliferative lesions, macular edema, local ischemia, or hard exudation invading the fovea are common causes of visual loss; proliferative vitreoretinopathy and tractive retinal detachment can cause severe visual loss. Visual loss is not a specific symptom of DR, fundus examination is more important. Hard exudation, wadding spot, retinal vascular disease, macular disease, vitreous disease, and optic neuropathy can be observed. In terms of microstructure, retinal capillaries have a special layer of cells, called pericytes, in addition to endothelial cells and basement membrane. In addition, they also have a contractile function, which can regulate local blood flow and vascular permeability of retinal capillaries, and inhibit the proliferation of endothelial cells through contact inhibition. The main pathological changes of DR include microvascular occlusion - sorbitol accumulates in vascular intimal cells to form high osmotic pressure, which makes intimal cells swell and increase in volume. The number of retinal capillary pericytes in diabetic patients decreased significantly, and then lost contact inhibition, which may lead to the formation of microangioma. The formation of microangioma is caused by the local weakening of the retinal capillary wall. Hard exudation: Hard exudates are yellow, yellowish white waxy spots with clear boundaries, which can be seen more or more. Sometimes it is arranged in a ring around one or several microvessels, and can be fused into large patches; some of them are dense near the vein, showing a white sheath shape. After that, the main part of the retina is the exudate from the outer layer of the retina. Soft exudate (cotton wadding spot): soft exudate is actually not exudate, but the capillary occlusion of the retinal nerve fiber layer, resulting in local nerve fiber obstructive necrosis. This spot indicates retinal ischemia. Performance for the white boundary fuzzy shuttle shape or irregular shape lesions, similar to cotton wadding, so also known as cotton wadding spots. When the eye ground appears with cotton wool spots, it indicates that DR is very serious. If a large number of them appear, it means that the disease is very active and may have entered the pre proliferation stage.

The increase in vascular permeability stimulates endothelial cells to synthesize vascular cell adhesion molecule-1 and promotes the adhesion and infiltration of leukocytes and monocytes to blood vessels. Age binds to endothelial cells, causing vasoconstriction, such as endothelin-1. Age can produce oxidative stress, increase the level of NF - $\varkappa$ B, induce no, reduce or inactivate endothelial production, enhance coagulation activity, and cause retinal hemodynamic abnormalities. Age can also induce apoptosis of pericytes. However, in hyperglycemia, especially in patients with diabetes, protein glycosylation is more likely to occur due to the increase in blood glucose. The gap is deposited in endothelial cells, pericytes, and basement membrane, thus activating white blood cells. The latter may block retinal capillaries due to abnormal attachment and infiltration of retinal capillaries. Under the effect of high glucose or age, cultured retinal capillary pericytes can synthesize collagen skin, promote arteriosclerosis of diabetic

arterioles, cause microvascular occlusion, ischemia, and hypoxia, and accelerate the formation of new blood vessels. Growth factors can bind to the corresponding receptors and promote cell mitosis and induce angiogenesis. Deoxyglucuronone is a diallyl compound closely related to the nonenzymatic glycosylation of proteins. It is a highly active 2-light aldehyde compound. It can cross-link with protein. There are many kinds of 3-DG reductase in tissues and organs, which can rapidly and effectively reduce 3-DG to 3-deoxyfructose, which can be excreted from urine. In addition, the content of 3-DG in the normal human body is very low. However, serum 3-DG was significantly increased in patients with diabetes. Cell growth factor (CGF) is a protein or multiple skin that can promote cell division, proliferation and, differentiation in vivo and in vitro. As a molecular signal or mediator, it can combine with specific cell receptors to promote cell growth or proliferation.

Puerarin

Puerarin is an active isoflavone extracted from Pueraria lobata or dried root of Pueraria lobata. In recent years, it has been used in the treatment of type 2 diabetes and its complications. Animal and clinical trials have shown that puerarin has many pharmacological effects, such as dilating coronary artery, antioxidation, lowering blood glucose, increasing insulin receptor sensitivity, and so on. Diabetes is caused by the relative or absolute insufficiencies of insulin secretion and the decrease of insulin sensitivity of target tissue cells. Oxidative stress refers to the process in which the body produces too many reactive oxygen species (ROS) or has a metabolic disorder, which exceeds the ability of the endogenous antioxidant defense system to clear reactive oxygen species. The mechanism of ROS production induced by high glucose and high fat is that glucose, fat, and other nutrients produce pyruvate and fatty acid in the cytoplasm. These substances selectively enter the mitochondrial matrix from the cytoplasm and degrade into two-carbon units after a series of changes.

They combine with coenzyme A to form acetyl coenzyme A and enter the tricarboxylic acid cycle; hydrogen separated from the tricarboxylic acid cycle passes through the respiratory chain (an electron transport system on the inner membrane of mitochondria) and finally enters oxygen. Diabetic retinopathy (DR) is a late complication of diabetes. It is one of the main causes of blindness and also a microvascular disease. Pericytes are important cell members that regulate retinal vascular perfusion. The damage of pericytes in diabetic patients can lead to changes in retinal hemodynamics, including abnormal self-regulation of retinal blood flow. Pericyte loss is another early change of DR, which is related to the formation of microaneurysm. Another pathological feature of DR is thickening of the capillary basement membrane and the deposition of extracellular matrix. This feature may lead to abnormal retinal hemodynamics, including abnormal blood flow self-regulation. Retinal leukocyte block may also play an important role in the pathological process of Dr. Granulocytes are large in size and stable in the cytoplasm. They have a natural chemotactic effect on vascular endothelium and can produce toxic superoxide radicals and proteolytic enzymes. The increase in leukocyte stagnation in diabetic patients affects retinal endothelial function, retinal perfusion, angiogenesis, and vascular permeability. Especially in patients with diabetes mellitus, granulocytes rarely deform and have a high activation rate, which may be related to the nonperfusion of retinal microvascular capillaries, endothelial cell damage, and vascular leakage. Diabetic hyperglycemia leads to an increase in the activity of the polyol pathway and finally leads to the increasean in sorbitol level. High glucose can activate the polyol pathway and induce pericyte apoptosis, which is a morphological change observed in the early stage of diabetes mellitus. The early loss of retinal pericytes may be due to the sensitivity of pericytes to polyols.

The main function of VEGF is to combine with tyrosine kinase receptors on vascular endothelial cells through paracrine or autocrine, so as to phosphorylate them, promote cell mitosis, make endothelial cells proliferate and swim, and induce neovascularization. Its receptors are mainly distributed in vascular endothelial cells, but not in smooth muscle cells, so they can specifically promote the mitosis of vascular endothelial cells. VEGFR is widely distributed in the membrane of retinal endothelial cells, and the expression of VEGFR is higher than that of normal retinal blood vessels, which indicates that VEGF in retinal endothelial cells reacts with high expression of VEGFR, which leads to tumor-related neovascularization. Doctors are an inflammatory disease. White blood cell abnormalities: increased leukocyte adhesion on the retina of diabetic patients. A leukocyte is a large group of cells, easy to adhere to vascular endothelium, and can produce toxic peroxides and proteolytic enzymes. Changes in

inflammation-related genes: up-regulation of genes encoding neutrophil adhesion in the retina of early diabetic rats. On the contrary, the expression of the gene encoding the decreased adhesion protein was down-regulated.

Expression of NF - κ B

The etiology and pathogenesis of diabetes are very complex. Oxidative stress caused by hyperglycemia and hyperlipidemia is a common mechanism of islet cell apoptosis and dysfunction, insulin resistance, and diabetic vascular complications. Some antioxidants can effectively improve the symptoms of diabetes. Antioxidant therapy has gradually become the focus of diabetes prevention and treatment. Large conductance potassium channels (BKCa), which depend on voltage and calcium, are widely distributed in the most important cells of the human body, especially in excitable cells, such as smooth muscle cells, adrenal chromaffin cells and ear hair cells, and play an important role in various physiological and pathological processes. Before abnormal fluorescence is found in fundus fluorescein angiography, abnormal microcirculation of the eyeball can be found by angiography. In most cells, glucose is converted to sorbitol by aldose reductase, which is converted to fructose by dehydrogenase.

Inositol is the precursor of phosphatidylinositol, which can be decomposed into two types of phosphatidylinositol (DG) and inositol triphosphate (IP3) under the action of the intracellular enzyme system. DG and IP3 are the second messengers necessary for the proliferation of pericytes. The lack of both can lead to pericyte apoptosis. The common examination methods are ophthalmoscope, fundus photography, fundus fluorescein angiography, ocular electrophysiology, visual contrast sensitivity, optical coherence tomography, etc. An ophthalmoscope is an early used fundus examination tool in clinics. The principle is to use the light near the lens to irradiate the fundus. The reflected light is collected and focused by the lens and observed by the inspector with the naked eye. The focal length can be adjusted. It has the advantages of low equipment cost, simple method, and noninvasive inspection. The disadvantage is that the subjectivity is relatively strong, and it is impossible to objectively record the image depending on the degree of care of the examiner. At present, the relatively backward areas still take this as the main means of inspection. Fundus photography is developed based on ophthalmoscopy. With the emergence of digital cameras and the continuous improvement of pixels, fundus photography can clearly record some lesions of the fundus by taking pictures. Related to it are hemangioma, punctate hemorrhage, sheet hemorrhage, hard exudation, cotton spots, and so on. In this examination, the pupil size of the patient should be checked first. If the pupil is not big enough, it is necessary to take pictures after mydriasis. If intraocular mydriasis is needed, the intraocular pressure should be checked. If the intraocular pressure is too high, it is not suitable for pupil dilation. The advantage of this method is that the fundus images can be recorded objectively and reliably. The disadvantage is that it cannot be used in patients with lens transparency, early primary retinopathy cannot be recognized, and photos are often unclear phenomena, need to further improve the pixel of the device, cannot see the formation of new blood vessels, so it is impossible to distinguish simple or proliferative. Fundus fluorescein angiography (FFA) provides a reliable basis for the early diagnosis and treatment of or. FFA can not only detect the early signs or the location and quantity of tumor microvessels but also reflect the changes of early organic microvessels before tumor microvessels, such as fluorescence leakage, capillary filling defect, tumor-like fluorescence, etc., which are helpful to the understanding of early detection of microvascular lesions and the development process of lesions. It can quantitatively estimate the range and size of the nonperfusion area of macular capillaries, diagnose the nature, extent, and degree of lesions, and closely estimate the location and activity of new blood vessels. It provides biological information for the treatment of or, especially the treatment of fundus laser, the evaluation of curative effect and prognosis. Before the occurrence of retinal microvascular disease, the pattern electroretinogram, flash electroretinogram, and oscillatory potential of diabetic patients have been abnormal, especially the prolongation of OPS latency is the most sensitive. EGR is called electroretinogram oscillatory potential. It is the most sensitive component of EGR to hypoxia, which can reflect the blood circulation state of the inner layer of the retina.

Retinal injury can increase the permeability of the retinal blood barrier, or the main early lesions often occur in the posterior pole of the retina, so this examination has important practical value for the early diagnosis of or. Under high glucose conditions, sorbitol increased significantly and inositol decreased significantly in RPE cells, which could be

inhibited by sorbini, an aldose reductase inhibitor. At the same time, the activation of the polyol pathway consumes more NADPH and reduces the adhesion of antioxidants. In addition, NADH can provide NADH oxidase energy, synthesize intracellular oxidation, and increase oxidative stress, leading to Dr. NF - κ B (NF - κ b) as a heterogeneous dimer of P50 / p65, which is a multidirectional nuclear transcription regulator. It binds to inhibitors of NF - κ B (PAB, IKB) and is inactive. They all have rel homologous regions similar to the coding products of rel gene (proto oncogene), including DNA binding domain, dimerization domain, and nuclear localization signal (NLS). Among the dimers of a protein family, P50 / rela is the most common. NF - κ B is a subunit composed of heterodimer p5op65. P50 is directly bound to DNA, and p65 has transcriptional stability. In the resting state, NF - κ B and IKB were destined and retained in the cytoplasm. In some stimuli, NF - κ B is released to the target gene of nuclear activation. The function of biogas protein is mainly to compensate for nuclear localization signal (NLS) NF - κ B and prevent the transfer of NF - κ B to human cells. In addition, it can prevent the junction and DNA nucleus of NF - κ B, and even isolate the complexity of NF - κ B and DNA. NF - κ B transcription factors include a classical activation mode and two nonclassical activation modes. The classic way of activation is the IKK (theixbkinase) pathway: stimulating signals to activate the special kinase signal complex IKK, catalyzing the phosphorylation of IKB protein, leading to IKB inactivation. PARP-mediated signal transduction and gene expression of inflammatory factors may be involved in the pathogenesis of retinopathy. Oxidative stress caused by various factors leads to DNA single-strand breaks, which activate PARP. PARP promotes the activation of NF - κ B and AP-1 expression, and increases the expression of NF - κ B and AP-1 dependent genes such as ICAM-1 also produce chemokines. Retinal capillary pericytes play an important role in maintaining the stability of retinal capillaries. Pericytes can regulate capillary blood flow, permeability, stability, and endothelial cell proliferation. Selective loss was the first characteristic lesion around Dr. Zhou in a high glucose environment, and NF - κ B was activated and Burlington was in midweek. Overexpression of Burlington protein can inhibit NF - κ B inhibitor. Burlington also induced overexpression and transfected NF - κ bp65 subunit. It is suggested that NF - κ B selectively activates Pro apoptosis in the early stage of diabetic retinopathy. A large number of activation of NF - κ B also revealed a model of retinal pericytes apoptosis induced by methylhexanediol. The application of NF - κ B inhibitor can inhibit apoptosis. Therefore, with the development of diabetes mellitus, hyperglycemia can activate NF - κ B, lead to apoptosis of periderm cells, normal retinal perfusion injury, aggravate hypoxia ischemia, and further activate NF - κ B, forming a vicious circle. Oxidative stress not only activates NF - κ B and Bcl-2 regulation, but also promotes Burlington translocation from cytoplasm to mitochondrial membrane, and regulates apoptosis of p53 and finally human endothelial cells.

Experiment

40 SD rats, SPF grade, 8 weeks old, weighing 190-240g, were provided by the animal center of a medical college. SPF animal room of the experimental animal center, feeding temperature is 25-27 °C, air circulation, humidity control is 49.0-75.4%, illumination is 12 hours, and conventional feed is fed.

Animal Feeding and Grouping

Environmental control: Breeding in SPF environment, closely monitoring room pollution and personnel flow, only full-time personnel can enter the room, full-time personnel must wear sterile surgical masks, hats, gloves, and long clothes. All items that come into contact with rats (feed and other items) must be disinfected. Drinking water must be treated to remove bacteria. Cages and bedding: cages must be cleaned once a week to ensure a clean and dry environment. Cages or other items cleaned with detergent or other detergents must be thoroughly cleaned. Bedding is dust-free hardwood chips and sharp pieces, ground corn stalks, or pine shavings. It should be disinfected before use and can be used only after disinfection. Diabetic rats have more urine and moist mattress, so they need to change the bed frequently. Therefore, diabetic rats are prone to infection, especially urinary tract infections and abdominal infections. Abdominal injection, subcutaneous injection, blood glucose measurement, and other invasive surgery should pay attention to disinfection. Food supply and water supply: make sure there is enough drinking water (insufficient drinking water can lead to the death of rats). When food and water are scarce, they kill each other and bite the same species. Therefore, the supply

of food and water should be adequate, preferably in two ways. Feeding: rat food must be sterilized, and it can enter the room after high-pressure steam sterilization. Drinking water: change the water bottle every day. The water bottle and water pipe must be cleaned and disinfected between use. Ten SD rats per cage were stained with fuchsin. The left is a single-digit stain. The front leg is No. 1, the left abdomen is No. 2, the left hind leg is No. 3, the top of the head is No. 4, the waist is No. 5, the tail base is No. 6, the right front leg of No. 7, the correct waist is No. 8, and the right hind leg is negative 9. The method of ten-digit staining was as follows: left forelimb No. 10, left abdomen No. 20, left hind limb No. 30, head No. 40, back No. 50, tail root No. 60, right forelimb No. 70. For example, mark a laboratory animal with No. 12, paint its left forelimb with magenta, and its left abdomen with picric acid (yellow).

Experimental Plan

To establish the diabetic retinopathy rat model: stz10g / l was prepared with 0.001mmol/l citric acid buffer, and the bacteria were filtered and sterilized. In the experimental group, STZ was injected into the left lower abdominal cavity. Blood glucose and urine glucose were measured after 72 hours. Diabetic rats with a blood glucose of 6.7 mmol / L and positive urine glucose were used as diabetic models. Diabetic rats were cultured for 3 months. The control group was given the same dose of citric acid buffer, and blood glucose was still measured before being killed. Preparation of STZ solution: add citric acid (FW: 210.14) 2.19 into 100ml double distilled water to prepare solution a; add sodium citrate (FW: 294.10) 2.949 to prepare solution B in 100ml double distilled water; STZ preparation solution: when using, mix solution a and B according to a certain proportion (1:1.32), pH value is determined by pH meter, adjust pH to 4.2-4.5, STZ is dissolved in 2% citric acid buffer solution and filtered by filter screen. UV disinfection is thick and practical. STZ preparation is thick and light proof. It should be injected as soon as possible to avoid decomposition. Operate on an ice bath. After intraperitoneal injection, the mouse tail was scratched with the little finger and ring finger of the left hand, and the neck of the mouse with the other three fingers, with the head slightly lower than the tail. With the right hand holding the syringe, the needle is almost parallel to a part of the subcutaneous part of the lower abdominal white line slightly to the right or left, the head needle is pushed about 3 mm, and then the injection needle is inserted into the skin of abdominal muscle at 45-degree angle, and enters the abdominal cavity through the abdominal muscle. When the needle passes through the abdominal muscle and enters the abdominal cavity, the resistance disappears. Fix the needle and retract the needle and plug. If there is no gas, liquid, or blood, inject the drug. Move gently to avoid stabbing abdominal organs. The principle and preparation of STZ induced diabetic animal model. STZ can selectively destroy islet P cells in some animals. Due to the destruction of a large number of P cells, the number of P cells is reduced, the secretion and synthesis of insulin are reduced, resulting in the disorder of glucose metabolism, resulting in increased blood glucose and diabetes symptoms. Rats and rats are usually used to make animal models. Foreign scholars reported that the rate of male rats was significantly higher than that of female rats. The preparation of animal models of type 1 diabetes mellitus and type 2 diabetes mellitus is related to the dosage of STZ injection. Two large doses of STZ can directly cause extensive destruction of islet P cells, which can lead to a type 1 diabetes mellitus model; when a small amount of STZ is injected, the surrounding tissues are not sensitive to insulin because only a part of the islet P cells is damaged, so the peripheral tissues are not sensitive to insulin, and high-calorie diet is given. At the same time, pathological and physiological changes are similar to the animal model of human type 2 diabetes. Model group: after 3 days of STZ injection, polydipsia, overeating, polyuria, sedentary, slightly poor spirit, weight, and blood glucose measurement were observed in the model group. If the fasting blood glucose was greater than 16.0 mmol, diabetic subjects with significantly increased urine and drinking water were included. Results thirty successful models were randomly divided into puerarin group (n = 10), model group (n = 10) and normal group (n = 10). Model group: the same amount of normal saline was injected once for 20 days. Normal group: normal feeding. Puerarin group: rats were intraperitoneally injected with Puerarin 10 mg / kg once a day for 20 consecutive days.

Immunohistochemical method: after 20 days, the rats were killed under anesthesia, and the eyeballs were gently removed and fixed. Send to the laboratory for fixation, dehydration, wax immersion, and embedding. The expression of NF - $\varkappa$ B was detected in the retina of diabetic rats. Paraffin embedding: the necks of 3 normal rats and

3 diabetic rats were directly removed and fixed in 10% formaldehyde. After fixation, the cornea, lens, and vitreous body were carefully removed, and the eyeball tissue was dehydrated with ethanol gradient. After dehydration, xylene was used to replace ethanol in tissues. Paraffin was soaked 3 times at 60 °C for 3 minutes each time. The tissue was put into the new paraffin to cool and solidify. After slicing, 4 cm slices were cut with a microtome. Immunohistochemical treatment: baking in a 60 °C incubator for 30 min, soaking LOMIN with xylene I, and soaking with xylene 11 for 10 min. After dewaxing, dehydrate with 95%, 85%, and 70% downward gradient alcohol, and soak in distilled water for 5 minutes. After dehydration, LOMIN was treated with 3% H202 at room temperature to inhibit endogenous peroxidase. Zmin was rinsed with distilled water 3 times; after antigen repair, 10% sheep serum was used to block the foreign protein for 15 min; when notehl antibody and NF - ϰ B antibody were diluted with PBS at 1:1000 and washed three times with PBS overnight, the temperature was at 40 °C. DAB was developed for smin, washed with distilled water, stained with hematoxylin 305, and dehydrated. The staining results were observed under a light microscope. The data were analyzed by spss-0 and spss-3. $P < 0.05$, the difference was statistically significant.

Experimental Results

Weight comparison

According to the Figure 1 and Table 1, the blood glucose of the successful model rats increased significantly 3 days after the establishment of the diabetes model, and there was obvious polydipsia, overeating, polyuria, emaciation, mental fatigue, and weight loss. There was no significant difference in the initial body weight among the three groups. The body weight of the normal group increased rapidly on the third day after injection ($P < 0.001$), and the difference gradually increased. After 3 weeks of treatment, the puerarin group was significantly higher than the normal group ($P < 0.05$).

Comparison of blood glucose

According to the Figure 2 and Table 2, there was no significant difference in blood glucose among the groups before modeling; there was no significant difference in blood glucose among the three groups after modeling for 3 days, 2 weeks and 3 weeks of treatment, but the difference was statistically significant compared with the normal group ($P < 0.001$).

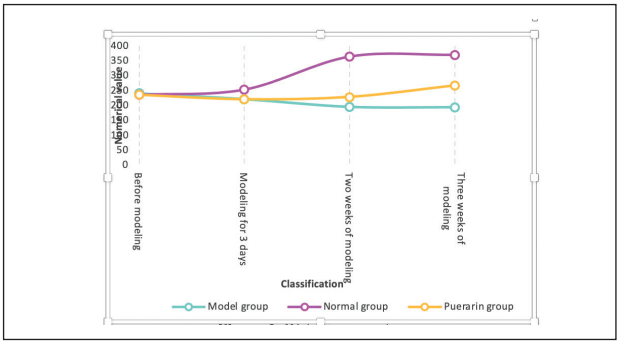

Figure 1: Weight comparison.

Group	Before modeling	Modeling for 3 days	Two weeks of modeling	Three weeks of modeling
Model group	241.12±4.98	222±7.22	196±11.54	194.74±15.28
Normal group	237±8.92	254.3±21.94	364.3±57.53	370.2±59.96
Puerarin group	236±11.80	221.13±13.38	229±44.6	267.60±48.88

Table 1: Weight comparison.

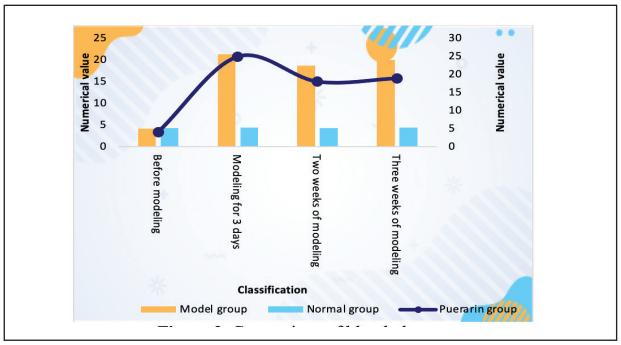

Figure 2: Comparison of blood glucose.

Group	Before modeling	Modeling for 3 days	Two weeks of modeling	Three weeks of modeling
Model group	4.15±0.28	21.37±5.06	18.71±1.56	20.05±2.87
Normal group	4.35±0.38	4.48±0.76	4.34±0.45	4.42±0.50
Puerarin group	4.13±0.25	24.94±5.11	18.04±3.36	18.93±3.94

Table 2: Comparison of blood glucose.

Protein expression

According to the Figure 3, the expression of PKC protein in the retina shows that PKC positive expression is located in the cytoplasm of ganglion cells and axons in the inner and outer plexiform layer. PKC protein was highly expressed in retinal ganglion cells and inner plexiform layer cells of SD rats. The results of the average gray value of PKC showed that the expression of PKC protein in the retina of the model group was significantly different from that of the normal control group ($P < 0.01$); the expression of PKC protein in puerarin group was lower than that in model group ($P < 0.01$).

Efficacy comparison

According to the statistical analysis of data, as shown in Figure 4, the treatment group was better than the control group in terms of symptom remission.

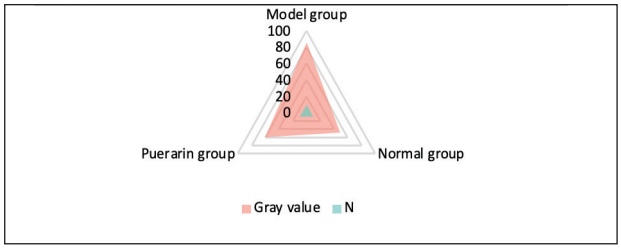

Figure 3: Protein expression.

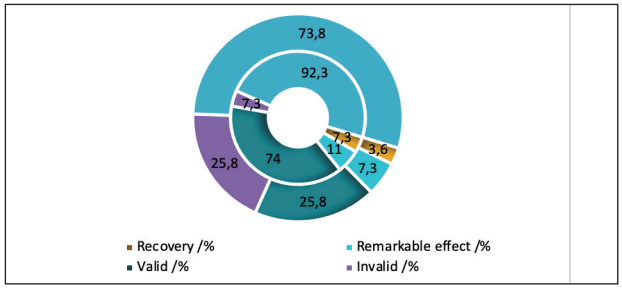

Figure 4: Efficacy comparison.

Discussion

DR can be divided into proliferative and nonproliferative according to the changes in fundus: nonproliferative is the early stage of the disease, which is limited to the retina, manifested as a microvascular tumor, hemorrhage, soft and hard exudates, retinal artery and venous disease; the value-added disease is characterized by neovascularization, and severe vitreous hemorrhage and retinal detachment can occur. Basic pathological process: chronic hyperglycemia, hyperlipidemia, and hypertension are the causes of diabetic retinopathy. The main manifestations of vascular disorders in DR were abnormal blood flow, increased vascular permeability, and low or no perfusion of capillaries. The marker of early diabetic retinopathy is the change in microvascular structure and cell composition. Visual contrast sensitivity is the ability of human eyes to distinguish fuzzy objects. It can display visual functions (such as vision) that cannot be displayed by traditional visual functions.

When there are a lot of organic substances in vitreous, vitreous hemorrhage, retinal strip hyperplasia, and retinal detachment are often caused, resulting in blindness or severe blindness. Therefore, based on strict control of diabetes, vitrectomy or vitrectomy can be used as appropriate to reduce retinal traction, close retinal holes, fill eyes with gas or liquid, and surround the outside of the eyeball to maintain or improve vision. If there is obvious macular traction, a vitrectomy should be performed in time even if there is no vitreous hemorrhage. The specific indications of vitrectomy are vitreous hemorrhage is difficult to absorb; laser treatment can be divided into diffuse photocoagulation and localized photocoagulation. The use of non-Street anti-inflammatory drugs, glucocorticoids, brain-derived nerve growth factors and other drugs can inhibit the inflammatory reaction, delay or reverse the pathological changes of retinal nerve cells, so as to achieve the purpose of prevention and treatment of diabetic retinopathy. Glucocorticoids reduce the production of prostaglandins by inhibiting arachidonic acid pathway and reduce vascular permeability by reducing VEGF. The mechanism of action of minocycline is to reduce the apoptosis of retinal nerve cells by reducing the expression of inflammatory cytokines and the reactivity of microglia. After Puerarin Treatment, hematocrit and plasma density decreased, while the whole blood, plasma, erythrocyte hardness, platelet count, total cholesterol, and glycerol decreased significantly before and after puerarin treatment.

Conclusion

Puerarin can improve vision and hemorheology. Puerarin and Xuesaitong can promote the absorption of bleeding and fundus leakage, reduce the fluorescence leakage of the fundus, and reduce the area of nonperfusion area of the fundus capillary. Puerarin can protect the diabetic retina by inhibiting PKC activation and NF - $\varkappa$ B expression, and reduce the expression of VEGF.

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