Heliyon 10 (2024) e24749

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Protective effect of Tetrandrine on optic nerve by inhibiting glial activation through NF-κB pathway

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ARTICLE INFO

Keywords:
Glaucoma
Glial cells
Neuroinflammation
Nuclear factor kappa B
Tetrandrine

ABSTRACT

Introduction: This study aimed to explore the effect and molecular mechanism of Tetrandrine (Tet) onlipopolysaccharide (LPS)-induceduveitis andoptic nerve injury *in vivo* and *in vitro*.
Methods: Uveitis was induced by LPS injected into the hindlimb foot pad of Wistar rats and was intervened by retroeyeball injection of Tet (100 nM, 1 μM or 10 μM).The anterior segment inflammation was observed by slit lamp. Tunelassay was used to detect the survival state of ganglion cells and nuclear layers of inner and outer. The detection of characteristic markers in different activation states of glial cells were performed by qualitative and quantitative test of immunofluorescence and western blotting. Also, western blotting was used to detect the expression of inflammatory factors in retina and the activation of nuclear factor kappa B (NF-κB) signal pathway. Meanwhile, routine blood test and function of liver and renal were performed.
Results: The ciliary hyperemia was obvious, and the iris vessels were dilated and tortuous in rats with LPS-induced uveitis. Tet-pretreated obviously elieved these symptoms. In addition, the dilation and hyperemia in Tet group were alleviated compared with LPS group, and the inflammatory scores in Tetgroup were significantly lower than those of LPS group. TUNEL Staining showed that the number ofretinal ganglion cell (RGCs) in Tetgroup was slightly less than that in normal group, but significantly more than that in LPS group, and the cells arranged orderly. Besides, the number of apoptotic cells was significantly less than that in LPS group. Tet reduced LPS-activated gliocyte in a dose-dependent manner. Tumour necrosis factor alpha (TNF-α), interleukin (IL)-1β, interferon gamma (γ-IFN) and IL-2 in retina were increased by LPS but decreased significantly viaTet-pretreatment. Moreover, LPS activate NF-κB signal pathway, while Tet efficiently inhibited this effect.Furthermore, injection of Tet did not damage theroutineblood, liver and kidney.
Conclusions: Retrobulbar injection of Tet significantly alleviatedLPS-induced uveitisand optic nerve injuryof rats by activating gliocyte and NF-κB signaling pathway.

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https://doi.org/10.1016/j.heliyon.2024.e24749
Received 23 January 2023; Received in revised form 9 December 2023; Accepted 12 January 2024
Available online 27 January 2024

1. Introduction

Uveitis is considered to cause damage to the uveal tissues of the eyes, which affects people of all ages, especially the youth and middle-aged people. Recurrent uveitis eventually contributes to serious complications including glaucoma [1,2].Glaucoma with irreversibilityis the second most common blind eye disease after cataract in the world [3]. Glaucoma is characterized by loss of retinal ganglion cells (RGCs) and visual field defects [4]. Intraocular pressure, ischemia and hypoxia, neurotrophic factor deprivation, gliocyteactivationandabnormal immune activation may be involved in RGCs damage [5]. In recent years, it has been found that the process of optic nerve injury is often accompanied by the migration, activation and proliferation ofgliocyte [6]. Gliocyte is activated and secrete proteolytic enzymes as well as proinflammatory factors, such as tumour necrosis factor alpha (TNF-α), interleukin (IL)-1β and IL-6 after acute and chronic retinal and optic nerve injury. The high expressions of these proinflammatory factors were found in both human glaucoma and lipopolysaccharide (LPS)-induced uveitisanimal models [7,8]. Therefore, inhibiting the inflammatory response mediated by gliocyte activation and improving the survival of RGCs may be effective measures for the prevention and treatment of uveitis and even glaucoma.

Tetrandrine (Tet) is an alkaloid isolated from the Traditional Chinese Medicine-*Tetrandra* or *Stephania*. More than half a century of researchs have shown that Tet has a wide and significant biomedical effect, such as anti-rheumatism, immunosuppression, anti-inflammatory, anti-allergy, anti-pyretic, analgesic, anti-cancer and other clinical therapeutic effects [9,10]. We have found that Tet is able toreduce intraocular pressure and resist RGCs damage and apoptosis caused by ischemia-reperfusion injury [11,12]. Therefore, we assumed that he Tet is capable ofinhibitingthe activation of immune cells in glaucoma and protecting optic nerve in LPS-induced uveitis model. In this study, we for the first time verified that Tet inhibited the activation of gliocyte and reduced the secretion of inflammatory factors of retina by inhibiting NF-κB signal pathway, thereby alleviating LPS-induced uveitis and RGC injury. These findings provided a promising therapeutic drug for uveitis and glaucoma treatment.

2. Materials and methods

2.1. Experimental animals

Wistar adult rats were purchased from Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China), male and female were not limited. The cage, feed, water and bedding were sterilized by high pressure in SPF aseptic condition, and replaced once every 3 days. 12 h of light and 12 h of darkness were used to maintain the physiological stability of these rats. The experimental process of this study was carried out in strict accordance with the Association for Research in Vision and Ophthalmology, and the Medical Experimental Animals of the Chinese Academy of Traditional Chinese Medicine.

2.2. Animal processing

Tetrandrine (Tet) was bought from MCE (HY-13764; USA). To obtain stock solution (10 mM), the powder was dissolved in 50 % PEG300 (MCE, USA) and 50 % saline (MCE, USA) in order. The adult male Wistar rats were randomly divided into each experimental groups. Each group contains 6 rats. After anesthesia using pentobarbital sodium, rats were retrobulbarly injected in with 100 nM, 1 μM and 10 μM Tet (Sigma, USA, No. T2695) 100 μL. Phosphate Buffer Saline (PBS) was used as control. LPS (Sigma, USA, No. L2630, 1 g/L) was injected into the hindlimb foot padto activate intraocular inflammation in rats, and PBS was used as the control. 24 h after LPS injection, the rats were euthanized, the right eye was isolated for subsequent analysis and the blood from retina was taken for routine blood test, liver and renal function test. IOP measurements were measured using rebound tonometry (Tonolab tonometer; USA). Before measurement, 0.5 % proparacaine hydrochloride was topically administrated to ocular surface while the rats were awake. The IOP measurement for each rat was repeated 5 times and averaged. All IOP measurements were conducted within 1 h to avoid diurnal discrepancy.

2.3. TUNEL staining

The retina was paraffin sectioned, and then the slices were dewaxed and hydrated. TUNEL Staining was performed according to the instructions of TUNELlBrightGreen Apoptosis Detection Kit (Vazyme, China, No. A112-01). Briefly, slices were pretreated with Protein K at room temperature for 20min, and then dropped 1xequilibration buffer for 30min after PBS cleaning. After that, dropwiseaddedTdT incubation buffer and incubated in a wet box at 37 °C for 60 min, and then 4′,6-diamidino-2-phenylindole (DAPI) solution was used for staining and incubated in dark for 2 min, and observation under the fluorescence microscope. The apoptotic cells in were labeled as green, fluorescent spots.

2.4. Immunofluorescence staining

Paraffin retinaslices were dewaxed and hydrated. Serum was added and blocked in a wet box at 37 °C for 60min, wiping off the blockedsolution with filter paper, addingprimary antibody and incubated in a wet box at 4 °C overnight. After PBS cleaning, added the diluted fluorescent secondary antibody in the wet box and incubated in the dark at 37 °C for 60min, and after PBS cleaning, staining with DAPI solution andphotographed under fluorescence microscope. Glial fibrillary acidic protein (GFAP) rabbit polyclonal antibody was purchased from MDL Companyand goat anti-rabbit IgG H&L (Alexa Fluor ® 488) was obtained from Abcam (ab150077).

Table 1
Scoring clinically in uveitis animal model.

clinical manifestations	Level	Score
iris hyperemia	no	0
	mild	1
	moderate	2
	severe	3
anterior chamber exudation	no	0
	small	1
	lot	2
Anterior chamber empyema	no	0
	yes	1
pupil	normal	0
	closed	1

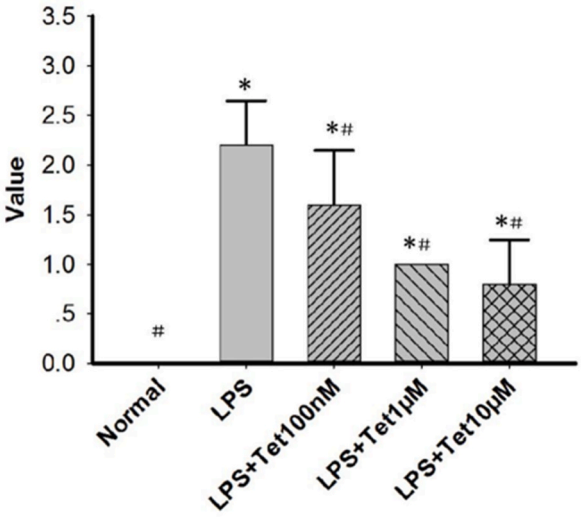

Fig. 1. Tet improved LPS-induced uveitis in rats. The inflammation score after indicated treatment was detected according to the score standard of uveitis animal model (n = 6 in each group).

2.5. Western blotting

The retinal tissues of rats isolated from right eye was added into protein extraction buffer (50 mM pH 7.4 Tris-HCl, 150 mM NaCl, 1 % NP-40) and the homogenate was ground in ice bath. Then the mixture was centrifuged for 15 min at 4 °C (12000 rpm/min). Next, the supernatant was collected after centrifugation and the protein concentration was measured by BCA method. A total of 40 μg proteins were loaded in 12 % SDS-PAGE and was transferred onto NC membrane. The membrane was blocked with skimmed milk powder dissolved in Tris Buffered Saline Tween (TBST, 10 mM pH 7.4 Tris-HCl, 150 mM NaCl, 1 ml Tween-20) at room temperature for 60min, washed with Tris Buffered Saline (TBS), and then incubated overnight with diluted primary antibody at 4 °C. After washing with TBST, added diluted second antibody and incubated at room temperature for 60min. After that, the protein bands were visualized using Super ECL Detection Reagent according to the protocol of manufacturer.Integrin subunit alpha M (CD11b, 14-0112-82, Invitrogen Antibodies, USA, Rabbit, 1:1000), cytochrome *c* oxidase subunit II (COX-2, ab15191, Abcam, UK, Rabbit, 1:1000), mannose receptor C-type 1 (CD206, 18704-1-AP, Proteintech, USA, Rabbit, 1:1000), SRY-box transcription factor 3 (SOX-3, ab42471, Abcam, UK, Rabbit, 1:1000), TNFα (17590-1-AP, Proteintech, USA, Rabbit, 1:1000), IL-1β (16806-1-AP, Proteintech, USA, Rabbit, 1:1000), IL-2 (12-0251-82,Invitrogen Antibodies, USA, Rabbit, 1:1000), IFN-γ (14-7311-81, Invitrogen Antibodies, USA, Rabbit, 1:1000), P65 (8242, Cell Signaling Technology, USA,Rabbit, 1:1000), p-P65 (3033, Cell Signaling Technology, USA,Rabbit, 1:1000), P50 (ab32360, Abcam, UK, Rabbit, 1:1000), p-P50 (4806,Cell Signaling Technology, USA,Rabbit, 1:1000), component of inhibitor of nuclear factor kappa B kinase complex (IKKα, 2682, Cell Signaling Technology, USA,Rabbit, 1:1000), *p*-IKKα (2694, Cell Signaling Technology, USA,Rabbit, 1:1000), goat anti-rabbit-IgG-HRP (7074, Cell Signaling Technology, USA,Rabbit, 1:10000) were purchased from Cell Signaling Technology company.

2.6. Statistical analysis

SPSS 17.0 software was used for statistical analysis. The data of each group (n = 10) was metrological data, expressed in x ± s, and

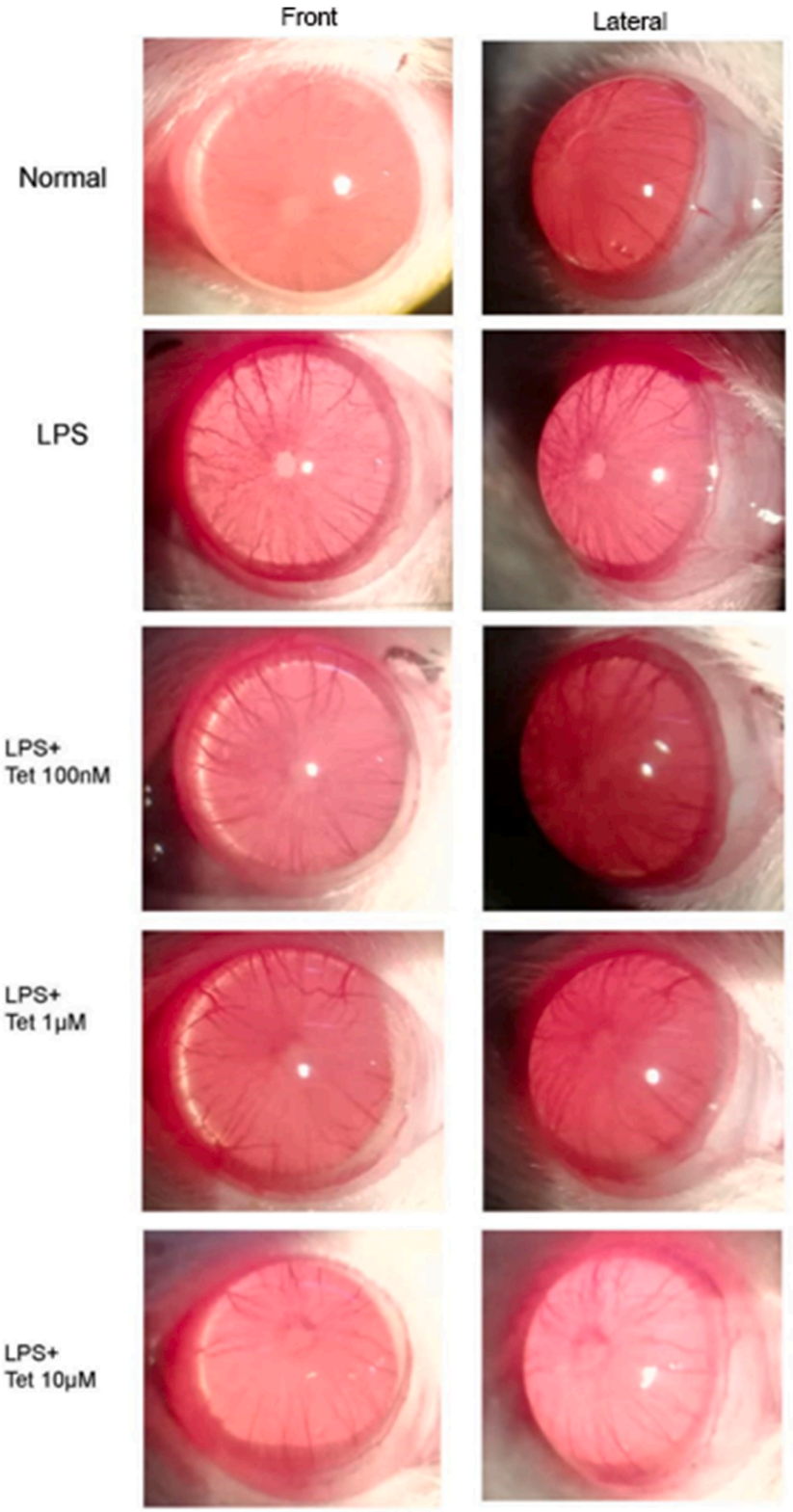

Fig. 2. Tet improved LPS-induced uveitis in rats. The inflammation stereophotography of uveitis rats was taken after indicated treatment.

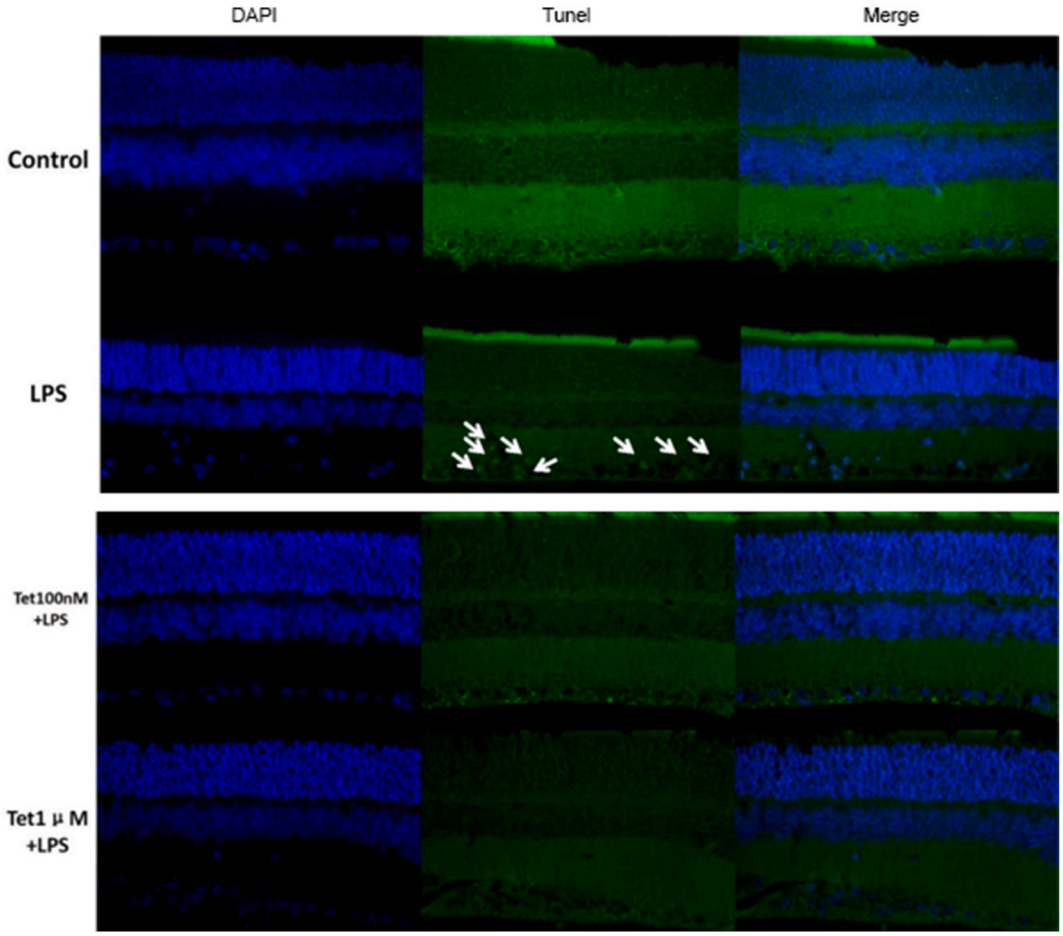

Fig. 3. Tet down-regulated apoptosis of retinal ganglion cells induced by LPS. The survival state of ganglion cells and nuclear layers was detected using Tunel staining assay after indicated treatment. Green: Tunel; Blue: DAPI. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

was analyzed by independent *t*-test. $p < 0.05$ was considered statistically significant.

3. Results

3.1. Tet improved LPS-induced uveitis in rats

To explore the role of Tet on uveitis, we established an LPS-induced uveitis model using Wistar rats, and the success rate of 24 h observation model was 100 %. The cracks on eyes at each time point were observed under the light. These results illustrated accumulation of inflammatory cells in the anterior chamber and flash of aqueous humor. In addition, mixed hyperemia and aqueous humor cells were found in all models. Besides, the inflammation score of each group was constructed according to the score standard of uveitis animal model (Table 1). The data showed that ciliary hyperemia, iris vasodilation and hyperemia of rats in LPS group was more obvious than that in control group. Additionally, the inflammation score and IOP of rats in LPS group was higher than that in control group ($P < 0.05$, Fig. 1, Fig. 2, Fig. S1). However, Tet-pretreatment partially reversed ciliary hyperemia, iris vasodilation, hyperemia and elevated inflammation score caused by LPS ($P < 0.05$, Figs. 1 and 2). Administration of Tet repressed the IOP in LPS-induced rat model in a dose-dependent manner (Fig. S1). Taken together, these findings suggested that Tet significantly alleviated LPS-induced uveitis in rats.

3.2. Tet down-regulated apoptosis of retinal ganglion cells induced by LPS

Inflammatory response induced by LPS causes the death of nerve cells in retina [13]. We employed TUNEL experiment and found that RGCs in control group presented larger nucleus and were orderly arranged with no obvious Tunel-positive cells. In LPS group, RGCs layer cells were sparse, and there were many small nuclear structures in inner plexiform layer, all cells in RGCs layer were

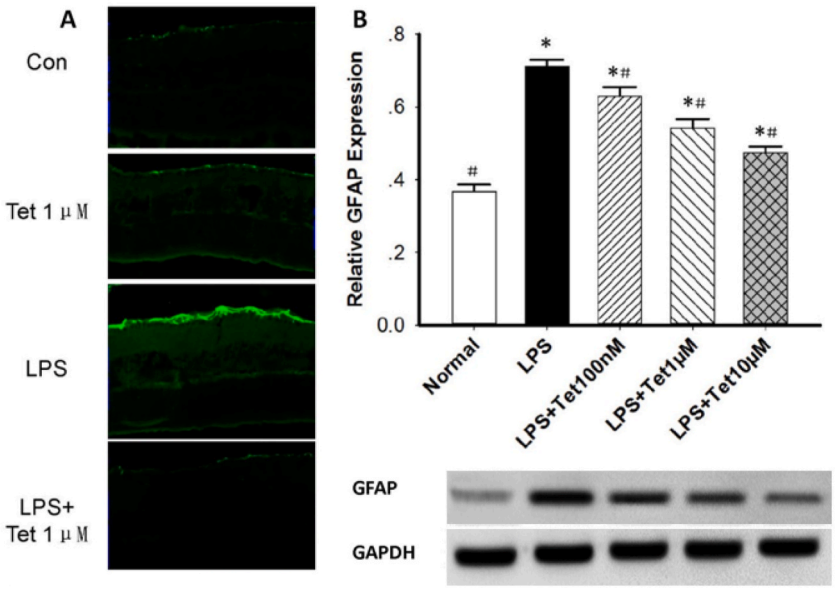

Fig. 4. Tet inhibited the activation of retinal gliocyte induced by LPS. A: Immunofluorescence localization of GFAP in retinal gliocyte; B: GFAP expression of Müller cellsin the retina detected via western blotting.

positive for apoptosis (white arrow). Nevertheless, Tet partly eliminated the role of LPS in RGC and reduced apoptotic cells, and the higher the concentration, the more obvious the effect (Fig. 3). Collectively, these results illustrated that Tet reduced LPS-induced apoptosis in RGCs in a dose dependent way.

3.3. Tet Inhibited the Activation of retinal gliocyte induced by LPS

LPS is able to activate Müller cells, the main gliocyte in retina [14]. To investigate whether Tet affects LPS-mediated activation of Müller cells, we detected the localization and expression of GFAP protein. As shown in Fig. 4A, GFAP is mainly in the retinal ganglion cell layer and inner plexus layer (Fig. 4A). Additionally, the protein level of GFAP in LPS group was significantly higher than that in control group (0.71 ± 0.02 vs 0.368 ± 0.019, $P < 0.05$), while Tet patially reduced GFAP expression in a dose dependent way (0.629 ± 0.025 , 0.541 ± 0.027, 0.473 ± 0.019 vs 0.71 ± 0.02, $p < 0.05$) (Fig. 4B). These data proved that Tet effectively inhibit LPS inducedMüller cellsin the retina.

LPS as the vitalcomponent of cell wall of Gram-negative bacteria contributes tosevereimmune response [15]. In addition to Müller cells, LPS has been found to activate otherwell-known microglial cells (expressing transmembrane protein 119, TMEM119 and CD11b), including their M1 type (expressing COX-2, inos myo-inositol-1-phosphate synthase, iNOS), M2a type (expressing Arg1 and mannose receptor C-type 1, CD206) and M2b type (expressing IL-1ra and SOCS3) [16]. Our results indicated that LPS dramatically increased the expression of CD11b(0.37 ± 0.004 vs 0.19 ± 0.002 , $p < 0.05$), COX-2(0.48 ± 0.012 vs 0.26 ± 0.0005, $P < 0.05$), CD206 (0.29 ± 0.007 vs 0.14 ± 0.0005 , $p < 0.05$) and suppressor of cytokine signaling 3 (SOCS3) (0.293 ± 0.0003VS 0.131 ± 0.002 , $p < 0.05$). However, 100 nM, 1 μM and 10 μM partly recoverd the protein level of CD11b (0.279 ± 0.014 vs 0.37 ± 0.004, 0.28 ± 0.005 vs 0.37 ± 0.004, 0.24 ± 0.007 vs 0.37 ± 0.004 $p < 0.05$), COX-2(0.33 ± 0.023 vs 0.48 ± 0.012, (0.28 ± 0.0025 vs 0.48 ± 0.012, 0.23 ± 0.013 vs 0.48 ± 0.012, $p < 0.05$) CD206 (0.206 ± 0.011 vs 0.29 ± 0.007 , 0.237 ± 0.007 vs 0.29 ± 0.007, 0.231 ± 0.008 vs 0.29 ± 0.007, $p < 0.05$) and SOCS3 (0.199 ± 0.011 vs 0.293 ± 0.0003, 0.21 ± 0.002 vs 0.293 ± 0.0003, 0.163 ± 0.003 vs 0.293 ± 0.0003, $p < 0.05$) increased via LPS in a concentration dependent way (Fig. 5). These data suggested that LPS dramatically activated microglia and and their three subtypes, whereas Tet-pretreatment partly reversed the activation.

3.4. Tet Inhibited the inflammatory response induced by LPS in retina

Gliocyteincluding microglia and Müller cells, are activated by the release of inflammatory factors such as TNF-α, IL-1β and γ-IFN that promote the production of NO and reactive oxygen species (ROS) and cause the damage of retinal nerve cells [17,18]. As shown in Fig. 6, LPS obviously increased the level of TNF-α (0.341 ± 0.001 vs 0.201 ± 0.006 , $p < 0.05$), IL-1β (0.267 ± 0.003 vs 0.091 ± 0.001, $p < 0.05$), γ-IFN (0.285 ± 0.002 vs 0.182 ± 0.001, $p < 0.05$) and IL-2 (0.252 ± 0.002 vs 0.173 ± 0.002, $p < 0.05$) in retina tissues. Nevertheless, 100 nM, 1 μM and 10 μM Tet treatment partially decreased the expression of TNF-α (0.255 ± 0.011 vs 0.341 ± 0.001, 0.232 ± 0.007 vs 0.341 ± 0.001, 0.24 ± 0.007 vs 0.341 ± 0.001, $p < 0.05$), IL-1β (0.2 ± 0.007 vs 0.267 ± 0.003, 0.2 ± 0.003 vs 0.267 ± 0.003, 0.162 ± 0.005 vs 0.267 ± 0.003, $p < 0.05$), γ-IFN (0.204 ± 0.012 vs 0.285 ± 0.002, 0.178 ± 0.003 vs 0.285 ± 0.002, 0.152 ± 0.007 vs 0.285 ± 0.002, $p < 0.05$) and IL-2 (0.188 ± 0.009 vs 0.252 ± 0.002, 0.194 ± 0.006 vs 0.252 ± 0.002, 0.141 ± 0.003 vs

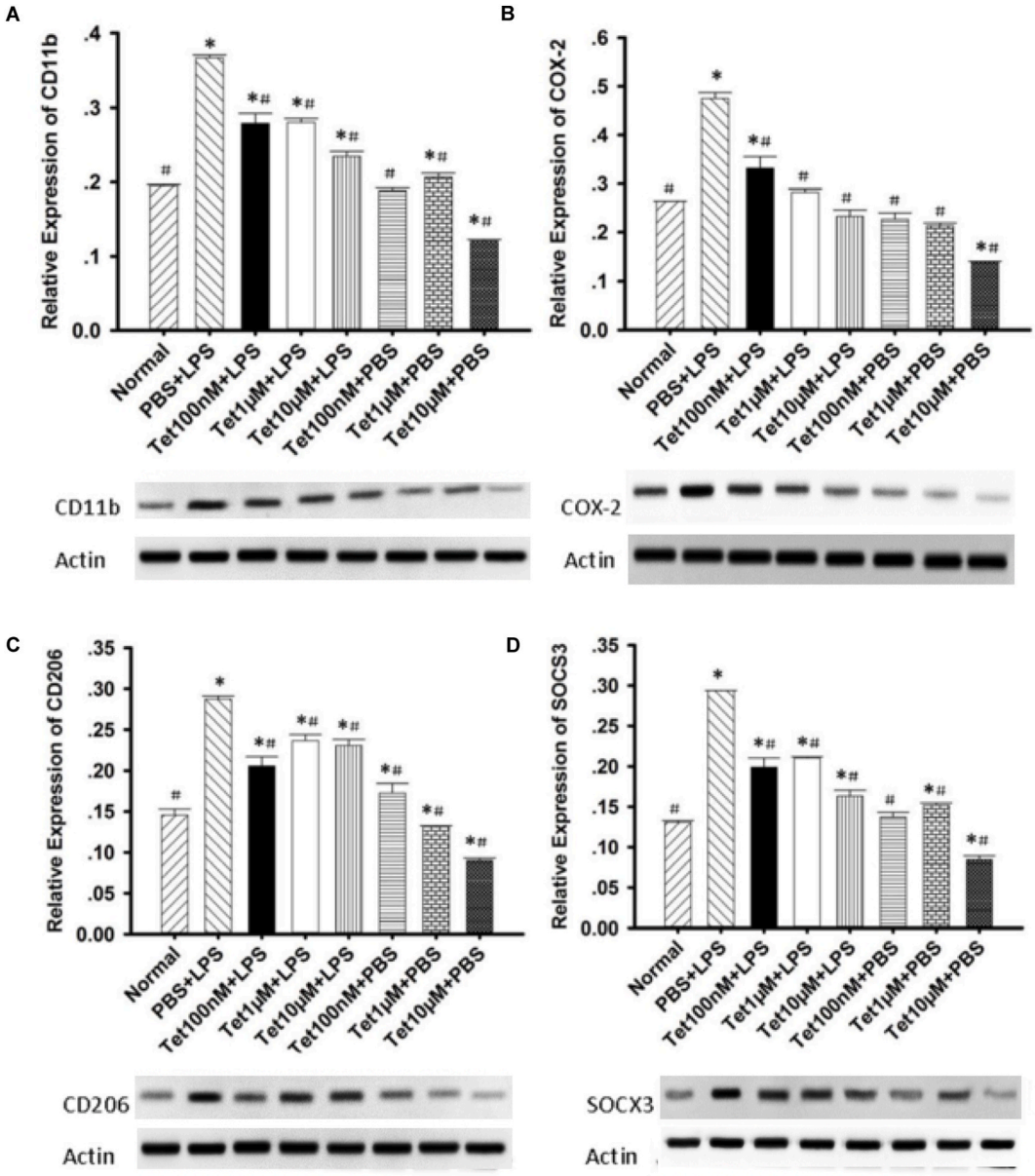

Fig. 5. Tet inhibited the activation of retinal gliocyte induced by LPS. The expression level of (A) CD11b, (B) COX-2, (C) CD206, and (D)SOCS3 proteins related to gliocyte activation. Experiments were repeated 3 independent times.

0.252 ± 0.002, $p < 0.05$) in a concentration denpendent manner (Fig. 5). Collectivelly, these findings revealed that LPS notably facilitated the inflammatory response in retina, while Tet partly counteracted the effect in a dose dependent way.

3.5. Tet Inhibited theActivation of NF- κBSignaling PathwayInduced by LPS

Subsequently, to explore whether NF-κB signaling pathway is involved in inflammatory response influenced by LPS or Tet, we measured the expression of proteins related to NF-κB pathway. The results showed that LPS significantly increased the content of *p*-IKKα and p-p65 but had no effect on p-p50, total IKKα, p65 as well as p50 protein levels (Fig. 7). Besides, treatment with 1 μM and 10 μM Tet partly inhibited the phosphorylation of p65 induced LPS (0.8 ± 0.06 vs 1.09 ± 0.1, $p < 0.05$, Fi. 7). Treatment with 1 μM and 10 μM Tet did not affect the phosphorylation of p50 ($P > 0.05$) but partially decreased the phosphorylation of IKKα ($p < 0.05$, Fig. 7). Thus, Tet efficiently suppressed the activation of NF-κB signaling pathway that induced by LPS.

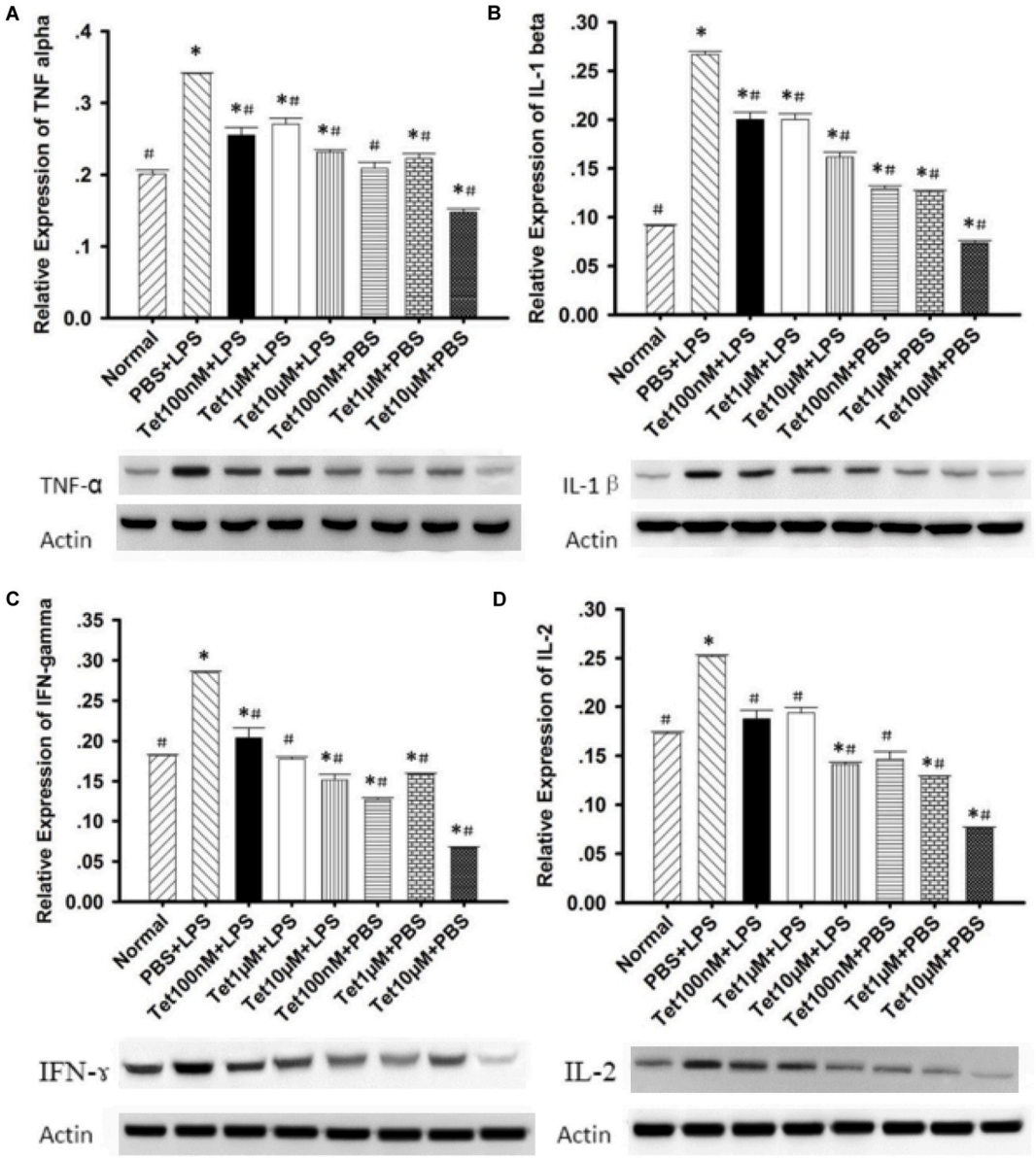

Fig. 6. Tet inhibited the inflammatory response induced by LPS in retina. The expression of inflammatory factors (A) TNF alpha, (B) IL-1 beta, (C) IFN-gamma, and (D) IL-2 in retina detected by western blotting. Experiments were repeated 3 independent times.

3.6. Routine blood examination

The results from routine blood biochemical test [19] showed that the total number of white blood cells (WBC) increased, but the total number of monocyte (MON) and lymphocyte (LYM) decreased after LPS injection compared with the rats in control group, which also reflected that LPS showed strong pro-inflammatory effect in the established rat model. Different concentrations of Tet reduced the inflammatory performance of routine blood test [20] in rats, but there was no significant difference compared with the normal group and LPS group. Tet had no significant effect on red blood cell (RBC) and hemoglobin (HGB), indicating that Tet had no significant effect on routine blood test in experimental animals (Fig. 8).

3.7. Examination of liver and kidney function

The results of liver function test showed that Tet had no abnormal effect on glutamic-pyruvic transaminase (ALT), only Tet at 1 μM caused the elevation of ALT, which indicated that Tet had no acute damage to liver function [21]. In clinical application, high alkaline phosphatase (ALP) indicates abnormal hepatobiliary system [22]. However, Tet did not cause elevation of ALP level. Thymine

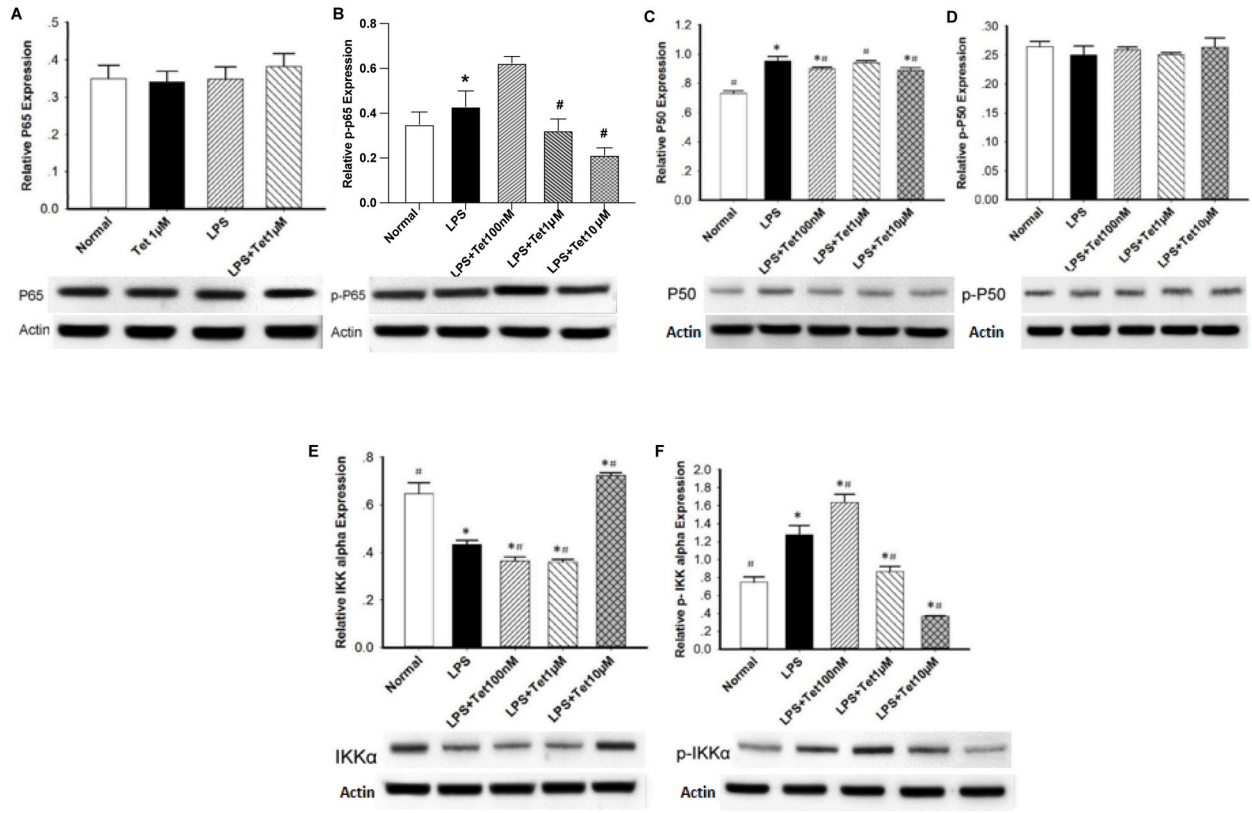

Fig. 7. Tet Inhibited the Activation of NF- κB Signaling Pathway Induced by LPS. The expression level of proteins related to NF-κB signaling pathway. (A) P65, (B) p-P65, (C) P50, (D) p-P50, (E) IKK alpha, and (F) IKK alpha. Experiments were repeated 3 independent times.

phosphorylase (TP) is closely related to angiogenesis, and the expression of TP in the plasma of tumor patients and tumor bearing animals is significantly increased [23]. Here, we showed that Tet does not promote the expression of TP (Fig. 9). The evaluation of kidney function [24] showed that the values of albumin (ALB), blood urea nitrogen (BUN), uric acid (UA), creatinine (CREA) in each group fluctuated little, basically without any significant difference, indicating that Tet had no significant acute damage to the kidney of experimental animals (Fig. 9).

4. Discussion

In this work, we found that Tet notably alleviated LPS-induced uveitis in rats and RGCs apoptosis caused by LPS. Additionally, Tet treatment efficiently inhibited the activation of gliocyte and inflammatory response caused by LPS in retina in a dose dependent manner (Fig. S2). Moreover, Tet exerted suppressive role in retina gliocyre by inhibiting the activation of NF-κB signaling pathway.

As a serious disease of the eye, uveitis has been found resulting in various complications including glaucoma [1,2]. Increasing evidence has illustrated that reducing intraocular pressure is no longer the only way to treat glaucoma, but also to prevent further damage to the visual function of patients from other aspects. Therefore, the concept of glaucoma optic nerve protection is proposed [25,26]. So far, more than 500 drugs have been used in neuroprotective therapy in different diseases, including free radical scavengers [27], apoptosis inhibitors [28], anti-inflammatory drugs [29], and gene therapy [30]. However, the role of Tet in uveitis and even glaucoma is unclear. In this study, we proved that LPS treatment induced uveitis in rats, which is consistent with previous reports [31]. In addition, we for the first time revealed that Tet pretreatment improved LPS-induced uveitis in rats efficiently.

Gliocyte are widely distributed in the central and peripheral nervous system. They maintain the balance of the body, form myelin sheath, and provide support and protection for neurons [32]. The main gliocyte are oligodendrocytes, astrocytes and microglia [33]. Microglia and Müller cells and astrocytes are the main gliocyte in retina, which play critical roles in the homeostasis of retinal neurons and retina healthy. Müller cells, as the main gliocyte in the retina, are involved in the metabolism of RGCs and the internal environment is stable, play an important role in the growth, damage, repair, and regeneration of RGCs. GFAP is a unique intermediate filament in glial cells, which is not expressed or rarely expressed in Müller cells of normal retina [34]. Under the pathological conditions of ischemia, retinal detachment, the high expression of GFAP in Müller cells is a marker of reactive gliosis [35,36]. It has been reported that activation of retinal glial cells can reduce the expression of glutamate receptor and lead to degeneration and death of RGCs [37]. Activated Müller cells can also aggravate RGCs damage by releasing tumor necrosis factor and NO [38,39]. In our present study, we discovered that LPS promoted RGCs apoptosis and increased the expression level of GFAP, while Tet partly inhibited

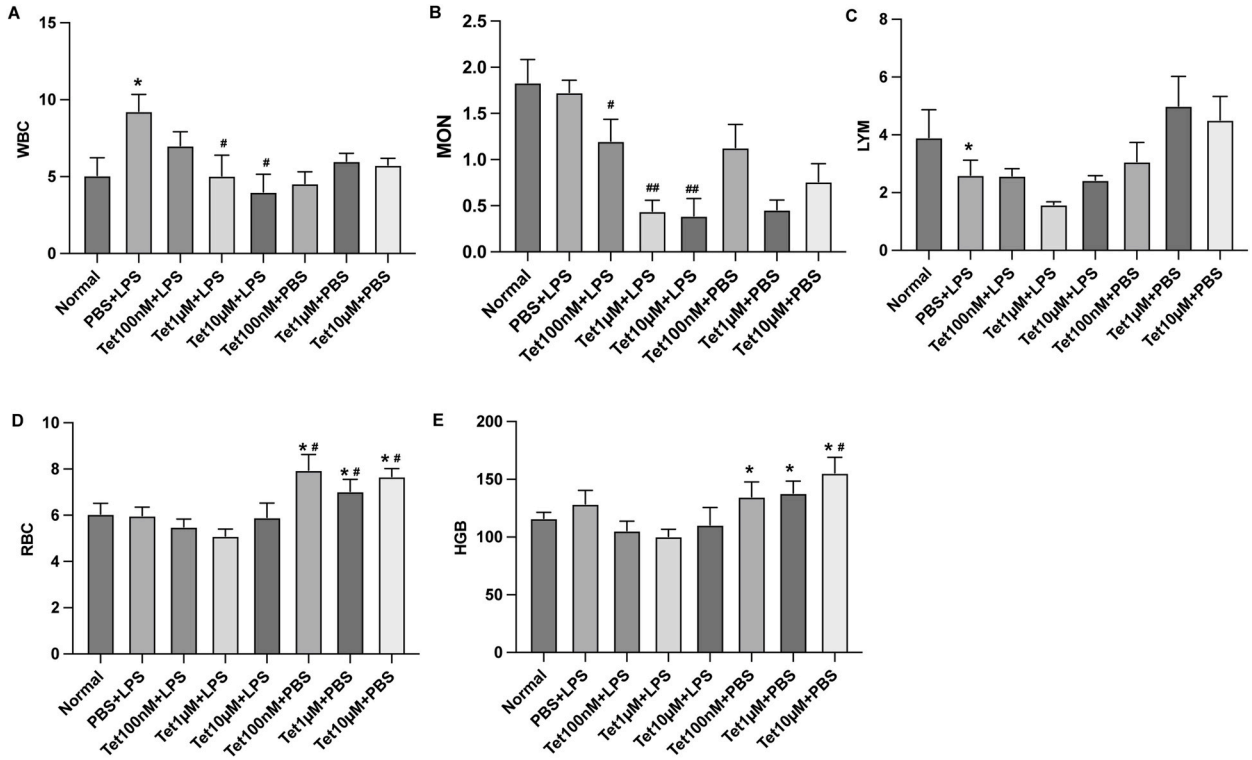

Fig. 8. Examination of routine blood. The Blood routine examination of related indicators. (A) White blood cells (WBC) increased, (B) monocyte (MON), (C) lymphocyte (LYM), (D) red blood cell (RBC), and (E) hemoglobin (HGB).

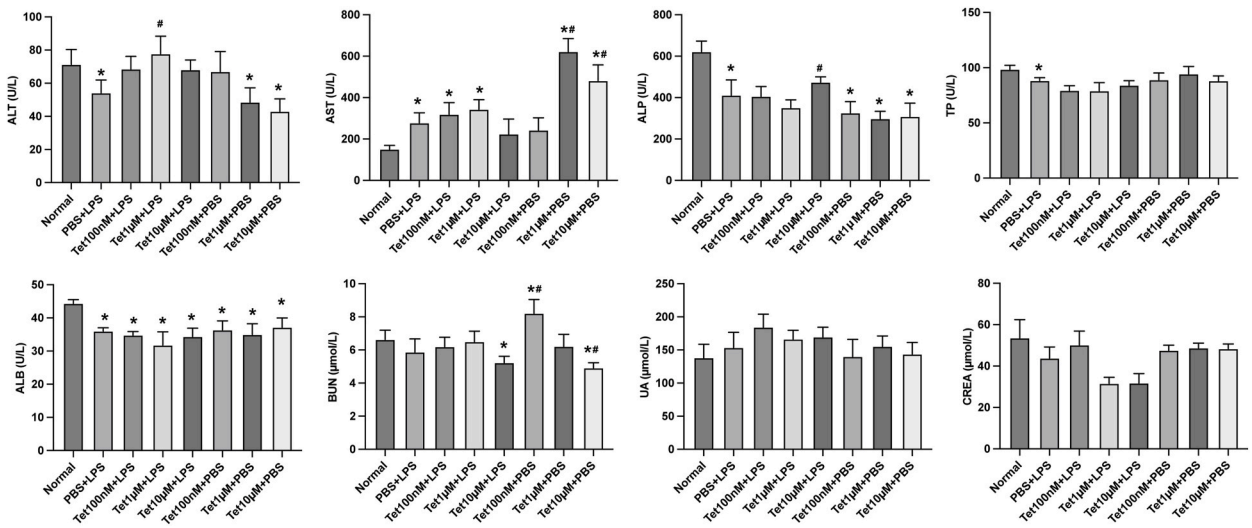

Fig. 9. Examination of liver and kidney function. Related indexes of liver and kidney function were detected.

apoptosis in RGCs and decreased GFAP protein in Müller cells in a dose dependent way. These findings verified that Tet suppressed RGCs apoptosis and Müller cells activation induced by LPS. At present, the reactive gliosis of Müller cells is considered to be a "double-edged sword". On the one hand, it actively resists damage and promotes the repair of neurons. On the other hand, it hinders tissue repair and nerve regeneration [40].

Microglia, as immune cells in the resident nervous system, play a key role in the formation of neuroinflammation [41]. By expressing CD11 b/C, D45 and C-X3-C motif chemokine receptor 1(CX3CR1), gliocyte detects the changes of homeostasis, and makes corresponding responses to exhibit defensive and neuroprotective functions [32]. When the nervous system is damaged, microglia is

activated and transforms to another phenotype, continues to proliferate and migrates to the injured site. Then activated microglia changes the expression of key enzymes as well as receptors and releases a variety of inflammatory factorssuch as NO, TNF-α and IL-6 [42]. As expected, our working confirmed that LPS significantly activated microglia and inflammatory response, while Tet eliminated these effects partly.

NF-κB is a multi-functional transcription factor, which can be expressed in almost all types of cells and plays an important role in the inflammatory response [43,44]. At the molecular level, inflammation is regulated by a variety of factors, such as ICAM-1 (ICAM-1), IL-8, IL-1, IL-2, TNF-α/β and NF-κB [45]. Some studies have shown that NF-κB can activate more than 500 genes related to inflammation [46]. Owing to its vital role, NF-κB-mediated signaling pathway is considered to be tightly associated with inflammation [47]. It is reported that NF-κB transcription factor exerts crucial effect on nervous systemiand the role of NF-κB in microglia is related to brain injury [48]. Prevous study also proved that LPS activated NF-κB signal can induce the production of inflammatory target protein COX-2 and PGE2, leading to cerebrovascular inflammation [49]. Consistently, this investigation verified that LPS dramatically activated NF-κB signaling pathway, whereas Tet treatment partially counteracted the effect. However, current findings based on the pre-treatment of Tet, further exploration of the therapeutic effects of Tet on uveitis using more *in vitro* and *in vivo* models is still needed.

In conclusion, this study suggested that Tet is a potential regetnt to reduce the inflammatory response related to uveitis and even glaucoma, reduce the production of retinal inflammatory factors, and protect retinal ganglion cells and optic nerve by inhibiting the activation of microglia and exhibited low toxicity and side effects. These findings may provide novel strategies for treatment of patients that diagnosed with uveitis and even glaucoma.

Statement of ethics

This study protocol was reviewed and approved by Ethic committee of China Acadamy of Chinese Medical Sciences, approval number 2020009.

Funding Sources

This work was funded by the National Natural Science Foundation of China (No. 81574032), Sanming Project of Medicine in Shenzhen (SZSM201812090), China Academy of Chinese Medical Sciences, Bureau of Human Resources and Social Security of Shenzhen and Finance Committee of Shenzhen, Construction Program for Inheritance Office of KANG Ze-feng, Famous TCM Experts of Shijingshan District in Beijing, Talent Project (Qihuang Project) and Qihuang Scholar for Heritage and Innovation of Traditional Chinese Medicine. The funding agencies have no role in the study design, collection, analysis and interpretation of data, the writing of this report, or on the decision to submit the article for publication.

Data availability

No data was used for the research described in the article.

CRediT authorship contribution statement

Weiyi Li: Conceptualization, Formal analysis, Investigation, Resources. **Jing Cao:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation. **Jian Liu:** Investigation, Validation. **Shuiling Chen:** Investigation, Validation. **Min Dai:** Investigation, Validation. **Mingming Zhang:** Data curation, Formal analysis, Methodology, Resources. **Xinyue Hou:** Data curation, Formal analysis, Methodology, Resources. **Jianquan Wang:** Data curation, Formal analysis, Methodology, Writing – original draft. **Zefeng Kang:** Conceptualization, Data curation, Investigation, Methodology, Resources, Supervision, Writing – review & editing.

Declaration of Competing interest

There are no conflicts of interest.

Acknowledgement

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e24749.

References

[1] K. Sabri, R.K. Saurenmann, E.D. Silverman, A.V. Levin, Course, complications, and outcome of juvenile arthritis-related uveitis, J AAPOS 12 (6) (2008) 539–545.
[2] B. Wang, M. Kasper, B. Laffer, Zu Meyer, G. Horste, S. Wasmuth, M. Busch, et al., Increased hydrostatic pressure promotes primary M1 reaction and secondary M2 polarization in macrophages, Front. Immunol. 11 (2020) 573955.
[3] H.A. Quigley, A.T. Broman, The number of people with glaucoma worldwide in 2010 and 2020, The British journal of ophthalmology 90 (3) (2006) 262–267.
[4] J.E. Morgan, G. Jeffery, A.J. Foss, Axon deviation in the human lamina cribrosa, The British journal of ophthalmology 82 (6) (1998) 680–683.
[5] M.C. Leske, Ocular perfusion pressure and glaucoma: clinical trial and epidemiologic findings, Curr. Opin. Ophthalmol. 20 (2) (2009) 73–78.
[6] S.G. Wohl, C.W. Schmeer, O.W. Witte, S. Isenmann, Proliferative response of microglia and macrophages in the adult mouse eye after optic nerve lesion, Investigative ophthalmology & visual science 51 (5) (2010) 2686–2696.
[7] V. Sivakumar, W.S. Foulds, C.D. Luu, E.A. Ling, C. Kaur, Retinal ganglion cell death is induced by microglia derived pro-inflammatory cytokines in the hypoxic neonatal retina, J. Pathol. 224 (2) (2011) 245–260.
[8] R.H. Shih, C.Y. Wang, C.M. Yang, NF-kappaB signaling pathways in neurological inflammation: a mini review, Front. Mol. Neurosci. 8 (2015) 77.
[9] L.J. Ho, D.M. Chang, T.C. Lee, M.L. Chang, J.H. Lai, Plant alkaloid Tetrandrine downregulates protein kinase C-dependent signaling pathway in T cells, Eur. J. Pharmacol. 367 (2–3) (1999) 389–398.
[10] Y. Xu, N.R. Aluru, Carbon nanotube screening effects on the water-ion channels, Appl. Phys. Lett. 93 (4) (2008) 43122.
[11] P. Huang, Y. Xu, R. Wei, H. Li, Y. Tang, J. Liu, et al., Efficacy of Tetrandrine on lowering intraocular pressure in animal model with ocular hypertension, J. Glaucoma 20 (3) (2011) 183–188.
[12] W. Li, C. Yang, J. Lu, P. Huang, C.J. Barnstable, C. Zhang, et al., Tetrandrine protects mouse retinal ganglion cells from ischemic injury, Drug Des. Dev. Ther. 8 (2014) 327–339.
[13] M.L. Aranda, D. Guerrieri, G. Piñero, M.F. González Fleitas, F. Altschuler, H.H. Dieguez, et al., Critical role of monocyte recruitment in optic nerve damage induced by experimental optic neuritis, Mol. Neurobiol. 56 (11) (2019) 7458–7472.
[14] L. Lorenz, S. Hirmer, A. Schmalen, S.M. Hauck, C.A. Deeg, Cell surface profiling of retinal muller glial cells reveals association to immune pathways after LPS stimulation, Cells 10 (3) (2021).
[15] Y. Cao, J. Chen, G. Ren, Y. Zhang, X. Tan, L. Yang, Punicalagin prevents inflammation in LPS-induced RAW264.7 macrophages by inhibiting FoxO3a/autophagy signaling pathway, Nutrients 11 (11) (2019).
[16] I.C. Hoogland, C. Houbolt, D.J. van Westerloo, W.A. van Gool, D. van de Beek, Systemic inflammation and microglial activation: systematic review of animal experiments, J. Neuroinflammation 12 (2015) 114.
[17] V. Murta, M.I. Farias, F.J. Pitossi, C.C. Ferrari, Chronic systemic IL-1beta exacerbates central neuroinflammation independently of the blood-brain barrier integrity, J. Neuroimmunol. 278 (2015) 30–43.
[18] J. Park, J.S. Min, B. Kim, U.B. Chae, J.W. Yun, M.S. Choi, et al., Mitochondrial ROS govern the LPS-induced pro-inflammatory response in microglia cells by regulating MAPK and NF-kappaB pathways, Neurosci. Lett. 584 (2015) 191–196.
[19] J. Chang, C. Liang, W. Wang, L. Yong, W. Mao, H. Yang, et al., Toxic effects of atrazine on immune function in BALB/c mice, Environ. Sci. Pollut. Res. Int. 28 (28) (2021) 37978–37994.
[20] X. Yin Pl, B.-Y. Liu, Y.-Y. Liu, W.-L. Fan, J.-H. Zhao, Preventive effects of low molecular weight heparin on formation of deep vein thrombosis by reducing D dimer values in patients undergoing spinal surgery, Eur. Rev. Med. Pharmacol. Sci. 22 (1) (2018) 229–237.
[21] S. Sookoian, C.J. Pirola, Liver enzymes, metabolomics and genome-wide association studies: from systems biology to the personalized medicine, World J. Gastroenterol. 21 (3) (2015) 711–725.
[22] D.H. Son, H.S. Ha, Y.J. Lee, Association of serum alkaline phosphatase with the TG/HDL ratio and TyG index in Korean adults, Biomolecules 11 (6) (2021).
[23] T.N. S Matsushita, T. Furukawa, T. Sumizawa, A. Tani, K. Nishimoto, S. Akiba, K. Miyadera, M. Fukushima, Y. Yamada, H. Yoshida, T. Kanzaki, S. Akiyama, The effect of a thymidine phosphorylase inhibitor on angiogenesis and apoptosis in tumors, Cancer Res. 59 (8) (1999) 1911–1916.
[24] X. Song, Z. Zhang, R. Zhang, M. Wang, D. Lin, T. Li, et al., Predictive markers of depression in hypertension, Medicine (Baltim.) 97 (32) (2018) e11768.
[25] J. Caprioli, Neuroprotection of the optic nerve in glaucoma, Acta Ophthalmol. Scand. 75 (4) (1997) 364–367.
[26] E. Yoles, M. Schwartz, Potential neuroprotective therapy for glaucomatous optic neuropathy, Surv. Ophthalmol. 42 (4) (1998) 367–372.
[27] X. Chen, A.S. Wang, Neuroprotective effects of free radical scavengers in stroke, Drugs Aging 24 (7) (2007) 537–546.
[28] K.K. Jain, Neuroprotection in cerebrovascular disease, Expert Opin Investig Drugs 9 (4) (2000) 695–711.
[29] M.J. Ammar, J. Hsu, A. Chiang, A.C. Ho, C.D. Regillo, Age-related macular degeneration therapy: a review, Curr. Opin. Ophthalmol. 31 (3) (2020) 215–221.
[30] V. Sudhakar, R.M. Richardson, Gene therapy for neurodegenerative diseases, Neurotherapeutics 16 (1) (2019) 166–175.
[31] X. Han, X. Chen, S. Chen, Q. Luo, X. Liu, A. He, et al., Tetramethylpyrazine attenuates endotoxin-induced retinal inflammation by inhibiting microglial activation via the TLR4/NF-kappaB signalling pathway, Biomed. Pharmacother. 128 (2020) 110273.
[32] A. Verkhratsky, V. Parpura, M. Pekna, M. Pekny, M. Sofroniew, Glia in the pathogenesis of neurodegenerative diseases, Biochem. Soc. Trans. 42 (5) (2014) 1291–1301.
[33] C.S. von Bartheld, J. Bahney, S. Herculano-Houzel, The search for true numbers of neurons and glial cells in the human brain: a review of 150 years of cell counting, The Journal of comparative neurology 524 (18) (2016) 3865–3895.
[34] A. Bringmann, I. Iandiev, T. Pannicke, A. Wurm, M. Hollborn, P. Wiedemann, et al., Cellular signaling and factors involved in Muller cell gliosis: neuroprotective and detrimental effects, Prog. Retin. Eye Res. 28 (6) (2009) 423–451.
[35] A. Wurm, I. Iandiev, S. Uhlmann, P. Wiedemann, A. Reichenbach, A. Bringmann, et al., Effects of ischemia-reperfusion on physiological properties of Muller glial cells in the porcine retina, Investigative ophthalmology & visual science 52 (6) (2011) 3360–3367.
[36] A. Bringmann, T. Pannicke, B. Biedermann, M. Francke, I. Iandiev, J. Grosche, et al., Role of retinal glial cells in neurotransmitter uptake and metabolism, Neurochem. Int. 54 (3–4) (2009) 143–160.
[37] K.R. Martin, H. Levkovitch-Verbin, D. Valenta, L. Baumrind, M.E. Pease, H.A. Quigley, Retinal glutamate transporter changes in experimental glaucoma and after optic nerve transection in the rat, Investigative ophthalmology & visual science 43 (7) (2002) 2236–2243.
[38] X. Lei, J. Zhang, J. Shen, L.M. Hu, Y. Wu, L. Mou, et al., EPO attenuates inflammatory cytokines by Muller cells in diabetic retinopathy, Frontiers in bioscience (Elite edition) 3 (2011) 201–211.
[39] K. Zeng, H. Xu, K. Chen, J. Zhu, Y. Zhou, Q. Zhang, et al., Effects of taurine on glutamate uptake and degradation in Muller cells under diabetic conditions via antioxidant mechanism, Molecular and cellular neurosciences 45 (2) (2010) 192–199.
[40] F. Ghosh, K. Johansson, Neuronal and glial alterations in complex long-term rhegmatogenous retinal detachment, Curr. Eye Res. 37 (8) (2012) 704–711.
[41] I. Glezer, A.R. Simard, S. Rivest, Neuroprotective role of the innate immune system by microglia, Neuroscience 147 (4) (2007) 867–883.
[42] P. M, D. Gl, Rationale for curcumin therapy in alzheimer's disease, ARC Journal of Neuroscience 1 (3) (2016) 7.
[43] B. Kaltschmidt, D. Widera, C. Kaltschmidt, Signaling via NF-kappaB in the nervous system, Biochim. Biophys. Acta 1745 (3) (2005) 287–299.
[44] P.A. Baeuerle, T. Henkel, Function and activation of NF-kappa B in the immune system, Annu. Rev. Immunol. 12 (1994) 141–179.
[45] C.M. Yang, C.S. Chien, L.D. Hsiao, S.F. Luo, C.C. Wang, Interleukin-1beta-induced cyclooxygenase-2 expression is mediated through activation of p42/44 and p38 MAPKS, and NF-kappaB pathways in canine tracheal smooth muscle cells, Cell. Signal. 14 (11) (2002) 899–911.
[46] S.C. Gupta, J.H. Kim, S. Prasad, B.B. Aggarwal, Regulation of survival, proliferation, invasion, angiogenesis, and metastasis of tumor cells through modulation of inflammatory pathways by nutraceuticals, Cancer metastasis reviews 29 (3) (2010) 405–434.

[47] W.W. Lin, M. Karin, A cytokine-mediated link between innate immunity, inflammation, and cancer, The Journal of clinical investigation 117 (5) (2007) 1175–1183.
[48] B. Kaltschmidt, C. Kaltschmidt, NF-kappaB in the nervous system, Cold Spring Harbor Perspect. Biol. 1 (3) (2009) a001271.
[49] R.H. Shih, C.M. Yang, Induction of heme oxygenase-1 attenuates lipopolysaccharide-induced cyclooxygenase-2 expression in mouse brain endothelial cells, J. Neuroinflammation 7 (2010) 86.