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EFFECT OF TRIGONELLINE IN A MODEL OF APOPTOSIS IN RAT RETINA

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ABSTRACT

Purpose: Determine the effectiveness of drop trigonelline and oral trigonelline (TG) as treatments for retinal apoptosis caused by N-methyl D-aspartate (NMDA) and to compare the effectiveness of TG drops with brimonidine tartrate (BT) drops.

Material and Method: We randomly divided rats into six groups of seven each. We didn't take any action to Group1. On the first day of the experiment (FDE), we administered intravitreal phosphate buffered saline (PBS) to Group2, the negative control, without administering any treatment. We administered intravitreal NMDA to Groups 3, 4, 5, and 6 on the FDE. Group3 (the positive control) didn't receive post-injection treatment. Group4 received oral TG, Group5 received TG drops, and Group6 received BT drops. All rats underwent histopathological and biochemical evaluations.

Results: TUNEL labeling revealed no significant changes between Groups 1 (1.21±0.08) and 2 (1.27±0.11), whereas Group 3 (12.52±2.82) had considerably higher indices ($p<0.001$). Compared to Group3, drop TG (4.67±1.23) and drop BT (4.26±0.97) significantly reduced apoptosis ($p<0.001$). MDA levels peaked in Group3 (3.23±0.85), with substantial reductions in all treatment groups ($p<0.05$). In Group3, SOD levels dropped a lot (14.6±4.01) compared to Group 1 (31.51±8.04, $p<0.001$), but oral TG, drop TG, and drop BT increased significantly (34.73±5.16, 38.46±3.60, 35.98±4.89, $p<0.001$). In Group3, iNOs protein expression increased by 32%, while the drop TG (~21%, $p=0.037$) and drop BT (~20%, $p=0.04$) groups showed substantial declines.

Conclusion: Our study found that the retinal neuroprotective and antioxidant efficacy of TG drops was comparable to that of BT drops.

Key Words: Neuroprotection , Retinal Apoptosis, Trigonelline

RAT RETİNASINDAKİ APOPİTOZİS MODELİNDE TRİGONELLİNE ETKİNLİĞİ

ÖZET

Amaç: N-metil D-aspartat (NMDA) ile indüklenmiş retinal apopitozis modelinde damla trigoneline ve oral trigonelinenin (TG) etkinliğini belirlemek ve TG etkinliğini brimonidin tartarat (BT) ile karşılaştırmak.

Materyal-Metod: Ratlar, her birinde yedi rat olacak şekilde rastgele altı gruba ayrıldı. Grup1'e herhangi bir işlem veya tedavi uygulanmadı. Deneyin ilk gününde (DİG) Grup 2'ye (negatif kontrol) intravitreal fosfat tamponlu salin (PBS) uygulandı ve sonrasında herhangi bir tedavi verilmedi. DİG'de Grup 3, 4, 5 ve 6'ya intravitreal NMDA uygulandı. Grup3'e (pozitif kontrol), enjeksiyon sonrası tedavi uygulanmadı. Grup4'e oral TG, Grup5'e damla TG ve Grup6'ya damla BT tedavisi verildi. Tüm rat gözlerinde histopatolojik ve biyokimyasal değerlendirmeler yapıldı.

Bulgular: TUNEL boyamasında, Grup 1 (1,21±0,08) ve 2 (1,27±0,11) arasında anlamlı bir fark olmadığı görüldü. Grup3'ün (12,52±2,82), Grup1'e göre yüksek düzeyde apopitotik indekse sahip olduğu izlendi ($p<0,001$). Grup3 ile karşılaştırıldığında damla TG (4,67±1,23) ve damla BT (4,26±0,97) gruplarında apopitozis anlamlı derecede azdı ($p<0,001$). MDA düzeyleri Grup 3'te yüksek izlenirken (3,23±0,85), tüm tedavi gruplarında önemli azalmalar görüldü ($p<0,05$). SOD düzeyleri grup 3'te (14,6±4,01) grup 1'e (31,51±8,04, $p<0,001$) göre anlamlı derecede azken, oral TG, damla TG ve damla BT gruplarında anlamlı düzeyde yüksek izlendi (34,73±5,16, 38,46±3,60, 35,98±4,89) , $p<0.001$). Grup3'te iNOs protein ekspresyonu %32 düzeylerindeyken, damla TG (~%21, $p=0,037$) ve damla BT (~%20, $p=0,04$) gruplarında ciddi azalmalar görüldü.

Sonuç: Çalışmamız, damla TG'nin retinal nöroprotektif ve antioksidan etkinliğinin, damla BT ile benzer düzeyde olduğunu tespit etmiştir.

Anahtar Kelimeler: Nöroproteksiyon, Retinal apopitozis, , Trigonelline

1.INTRODUCTION

Glaucoma, characterized by irreversible vision loss, primarily damages the retinal ganglion cells (RGCs). While high intraocular pressure (IOP) is known to increase the risk of

retinal nerve fiber loss, simply lowering IOP is not sufficient to stop the advancement of glaucoma in every patient. This highlights the necessity for alternative treatments (1,2). Recent advances have highlighted the role of neuroprotective strategies in glaucoma treatment, which aim to shield the optic nerve and RGCs from degeneration (3).

Glutamate excitotoxicity and oxidative stress play pivotal roles in the pathophysiology of glaucomatous neurodegeneration. The interaction of glutamate with NMDA receptors triggers a cascade of intracellular events, leading to elevated calcium levels and increased NOS activity. This process creates reactive oxygen species and turns on lysosomal enzymes, which in turn causes mitochondria to stop working and RGCs to die (4,5). We used an NMDA-induced excitotoxicity model in this study that accurately mimics these parts of glaucoma pathology. This model gives us a good way to look into RGC degeneration (6).

Given the challenges posed by glaucoma in terms of neuroprotection, TG emerges as a promising candidate. TG, derived from fenugreek seeds, is a water-soluble form of vitamin B6. It has many health benefits, such as lowering blood sugar and cholesterol, protecting nerve cells, stopping migraines, and improving brain function (7,8). Its diverse effects on the central nervous system suggest its potential usefulness in managing glaucoma, particularly due to its anti-apoptotic and antioxidant properties. Although TG has potential, our literature analysis could not find any extensive research on the effects of TG in ocular applications. We compared TG to BT, a medicine that effectively reduces intraocular pressure and enhances neuroprotective effects, to understand its potential (9-11). Our study aims to investigate the neuroprotective and antioxidant effects of TG when administered systemically and topically in a rat model of NMDA-induced excitotoxicity.

2. MATERIALS AND METHODS

Study Design and Animals

This study was conducted in accordance with the approval of the Fırat University Animal Experiments Lokal Ethics Committee Statement (Protocol No. 2019/96). The experiments were performed on 8–10-week-old Wistar albino male rats weighing 225–300 g, provided by the Fırat University Experimental Research Center. We housed the rats under a 12-hour light cycle (7:00 AM to 7:00 PM) and a 12-hour dark cycle (7:00 PM to 7:00 AM) at room temperatures of 22–25 °C. All groups had ad libitum access to water and food.

Groups

Group 1 (control group): No procedures were performed.

Group 2 (Negative Control Group): FDE, 2 µl of 0.1M PBS was administered intravitreally in each eye using a Hamilton syringe.

Group 3 (Positive Control Group): 2 µl of 160 nmol/µl NMDA solution was administered intravitreally in each eye using a Hamilton syringe.

Group 4 (Oral TG Group): In addition to administering NMDA, this group also received a daily dose of 100 mg/kg of TG orally via gavage for 21 days.

Group 5 (Drop TG Group): Similarly to Group 3, we administered NMDA and applied 20 mg/ml (1 mg per drop) TG eye drops morning and evening for 21 days.

Group 6 (Drop BT Group): Similarly to Group 3, we administered NMDA and applied Allergan ALPHAGAN-P 0.15% 5 ml eye drops morning and evening for 21 days.

In our experiments, we administered TG orally at 100 mg/kg per day for three weeks, as per Alzheimer's and Parkinson's models (12,13). We used a higher topical dose of 20 mg/ml for TG, compared to 1.5 mg/ml for the topical BT group, due to the absence of toxicity at high systemic doses.

Retinal Excitotoxicity Induction and Post-Operative Procedures

Anesthesia and analgesia were achieved using a combination of 50 mg/kg ketamine hydrochloride (Ketalar, Eczacıbaşı, Turkey) and 5 mg/kg xylazine hydrochloride (Rompun, Bayer, Turkey). We administered this regimen to all groups except the control group (Group 1). All rats received 1% proparacaine hydrochloride topically in both eyes after the intramuscular injection. To cause retinal excitotoxicity, 2 µl of 0.1 M PBS was injected intravitreally into both eyes of rats in Group 2 using a 30-gauge Hamilton syringe that was placed 1 mm behind the limbus. In the same way, rats in Groups 3, 4, 5, and 6 were given an intravitreal injection of 2 µl of 160 nmol/µl NMDA solution in the same part of their bodies. We administered antibiotic eye drops to all eyes post-injection to prevent infection.

On the 21st day of the experiment, we euthanized the rats and enucleated both eyes. We placed an orientation suture at the 12 o'clock position of the right eyes prior to enucleation to ensure consistent anatomical orientation during subsequent evaluations. The Department of Medical

Pathology received the right eyes for histopathological examination, while the Department of Medical Biochemistry processed the left eyes for biochemical analyses.

Histopathological Examination

Hematoxylin-Eosin Staining

We marked the right eyes of the rats with black ink on the temporal side, 2 mm anterior to the optic disk. Tissue samples from the marked areas were cut vertically into 3–4 μm slices and stained with hematoxylin and eosin to show all the tissues that had been removed.

TUNEL Staining

Sections of 3–4 μm thickness from formalin-fixed, paraffin-embedded tissues were dehydrated and then rehydrated several times. They were then incubated with 0.05% proteinase K for 10 minutes and 3% hydrogen peroxide for 5 minutes to stop the body's own peroxidase activity. The ABP Biosciences TUNEL Chromogenic Apoptosis Detection Kit treated the sections with equilibration buffer for 6 minutes and a TUNEL reaction mixture for 60 minutes at 37C after washing them with PBS. We further processed the tissues with anti-digoxigenin peroxidase for 30 minutes, developed them with diaminobenzidine (DAB), and counterstained them with Mayer's hematoxylin. Brown nuclear staining identified apoptotic cells, while normal nuclei appeared blue. We calculated the apoptotic index by calculating the ratio of apoptotic to total cells in 100 randomly selected cells at 40x magnification.

Immunohistochemical Evaluation of Caspase 3 and Brn3a

We put 3–4 μm thick slices on polylysine slides and used the Ventana Ultraview Universal DAB Detection Kit in a Ventana Benchmark Ultra system to treat them with Caspase 3 and Brn3a antibodies. We evaluated the staining intensity using an Olympus BX50 microscope at 40x magnification in 10 randomly selected fields. We scored the staining as follows: Grade 0 (no staining), Grade 1 (1-10% of cells positive), Grade 2 (11-30%), Grade 3 (31-50%), and Grade 4 (over 51% positive cells) (14).

Evaluation of Biochemical Samples

Preparation and Homogenization of Tissue Samples

We rinsed eye tissue samples with 0.9% cold sodium chloride (NaCl) at +4C and blotted them dry. We homogenized the tissues in a 0.01M PBS solution (dilution 1/10) at 16000 rpm

for 4 minutes, centrifuged at 5000 xg for 1 hour at +4 °C, and collected and stored the supernatants at -80 °C until further analysis.

Protein Quantification

We measured the protein concentrations in the supernatants using the Qubit Fluorometer (Invitrogen, USA) and the corresponding protein assay kit. The Lowry method, based on the colorimetric reaction of protein molecules with the Folin-Phenol reagent in an alkaline environment, also determined the total protein content (15).

SOD Activity Measurement

We quantified the SOD levels in the supernatants using an enzyme-linked immunoassay (ELISA) with a rat SOD ELISA kit (Andy Gene Biotechnology Co., Ltd.). We measured the absorbance at 450 nm using an EPOCH 2 microplate reader (BioTek Instruments, Inc., USA). We expressed the SOD concentration in ng/L, maintaining a sensitivity of 1.0 ng/L and an intra-assay coefficient of variation (CV) below 10%.

MDA Level Determination

Ohkawa et al (16) described a method for measuring MDA levels, which involved the spectrophotometric detection of a pink thiobarbituric acid reactive substance at 532 nm. This measurement reflects lipid peroxidation levels under specific conditions (acidic pH of 3.5 and aerobic conditions). We normalized the results to protein concentration and reported them in nmol/mg protein.

Western Blot Analysis for iNOs Protein Levels

Electrophoresis separated the proteins and transferred them to a nitrocellulose membrane. We then blocked the membrane to prevent non-specific binding and incubated it with specific antibodies targeting iNOs. We visualized the proteins using appropriate detection methods, ensuring a specific and accurate protein identification.

Statistical Analysis

We presented the data as mean and standard deviation. We conducted statistical analyses using SPSS software, version 22. We assessed differences between groups using a one-way ANOVA, followed by a post hoc Tukey test to compare mean values. A p-value of less than 0.05 was considered statistically significant.

3. RESULTS

TUNEL Staining Results

The mean TUNEL apoptotic indices were 1.21 ± 0.08 for group 1, 1.27 ± 0.11 for group 2, 12.52 ± 2.82 for group 3, 10.13 ± 2.04 for oral TG, 4.67±1.23 for drop TG and 4.26±0.97 for drop BT. We observed no significant statistical difference between groups 1 and 2 ($p > 0.05$). The TUNEL apoptotic index in group 3 was significantly higher than in group 1 ($p < 0.001$), while indices in the drop TG and drop BT groups were significantly lower than in group 3 ($p < 0.001$ for both) (Table 1). We found no significant difference ($p > 0.05$) between group 3 and the oral TG group(Figure 1).

	Group 1	**Group 2**	**Group 3**	**Oral TG**	**Drop TG**	**Drop BT**
TUNEL (Apoptotic Index %)	1,21 ± 0.08	1,27±0.11[b,c]	12,52±2.82[a]	10,13±2.04[a,d]	4,67±1.23[b,c]	4,26±0.97[b,c]
Caspase 3 Score	0,11± 0.00	0,12±0.00[b,c]	1,5±0.57[a]	1,17±0.40[a,d]	0,43±0.53[b,c]	0,5± 0.54[b,c]
Brn3a Score	3,33± 0.51	3± 0.00[b,c]	2.25 ± 0.50[a]	2,75± 0.40[a,d]	3,43±0.53[b,c]	3,71±0.48[b,c]

Table 1. TUNEL, Caspase 3 and Brn3a staining levels (a: $p<0.05$ compared to the group 1; b: $p>0.05$ compared to the group 1; c: $p<0.05$ compared with group 3; d: $p>0.05$ compared with group 3).

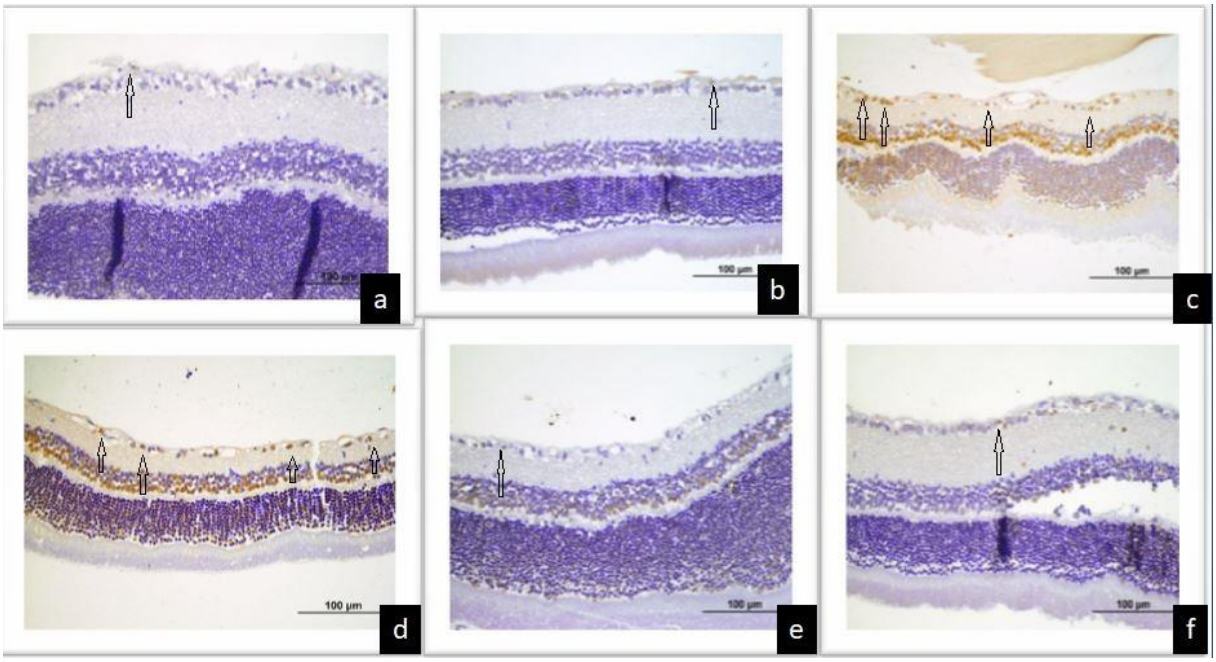

Figure 1. Representative images of TUNEL staining in retinal sections from different experimental groups. (a) Group I and (b) Group II display few apoptotic cells, as indicated by the arrows. In contrast, (c) Group III and (d) Group IV exhibit increased numbers of apoptotic cells (arrows). Notably, (e) Group V and (f) Group VI show a significant reduction in the number of apoptotic cells following treatment, as highlighted by the arrows.

Caspase-3 Results

The mean caspase-3 scores were 0.11 ± 0.00 for group 1, 0.12 ± 0.00 for group 2, 1.5 ± 0.57 for group 3, 1.17 ± 0.40 for oral TG, 0.43 ± 0.53 for drop TG, and 0.5 ± 0.54 for drop BT (Table 1). Caspase-3 scores in group 3 were significantly higher than in group 1 ($p < 0.001$). Scores were significantly lower in the drop TG and drop BT groups compared to group 3 ($p < 0.001$ for both). Group 3 and the oral TG group showed no significant difference ($p > 0.05$) (Figure 2).

Brn3a Results

The mean Brn3a scores were 3.33 ± 0.51 for group 1, 3 ± 0.00 for group 2, 2.25 ± 0.50 for group 3, 2.75 ± 0.40 for oral TG, 3.43 ± 0.53 for drop TG, and 3.71 ± 0.48 for drop BT (Table 1). We observed no significant difference between groups 1 and 2 ($p > 0.05$). Group 3 showed significantly lower Brn3a scores compared to Group 1 ($p < 0.001$). Brn3a scores were significantly higher in the drop TG and drop BT groups compared to group 3 ($p < 0.001$ for both)(Figure 3).

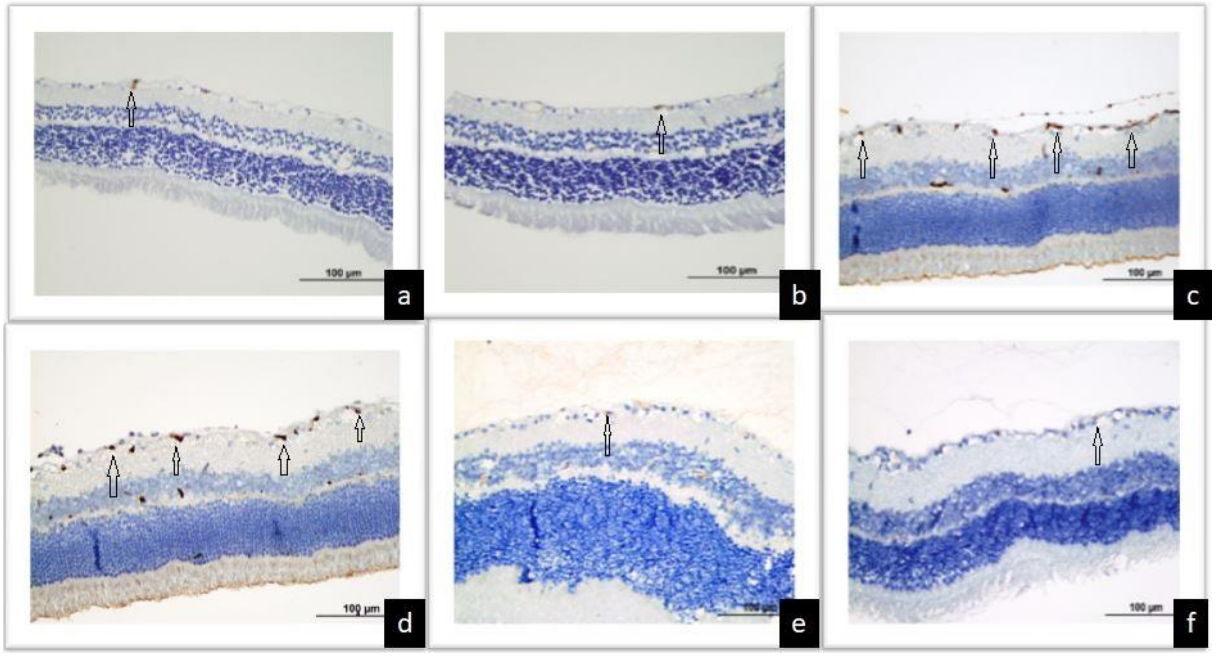

Figure 2. Representative images of caspase 3 staining in retinal sections from different experimental groups. (a) Group I and (b) Group II display few caspase 3 positive cells, as indicated by the arrows. In contrast, (c) Group III and (d) Group IV exhibit increased numbers of caspase 3 positive cells (arrows). Notably, (e) Group V and (f) Group VI show a significant reduction in the number of caspase 3 positive cells following treatment, as highlighted by the arrows.

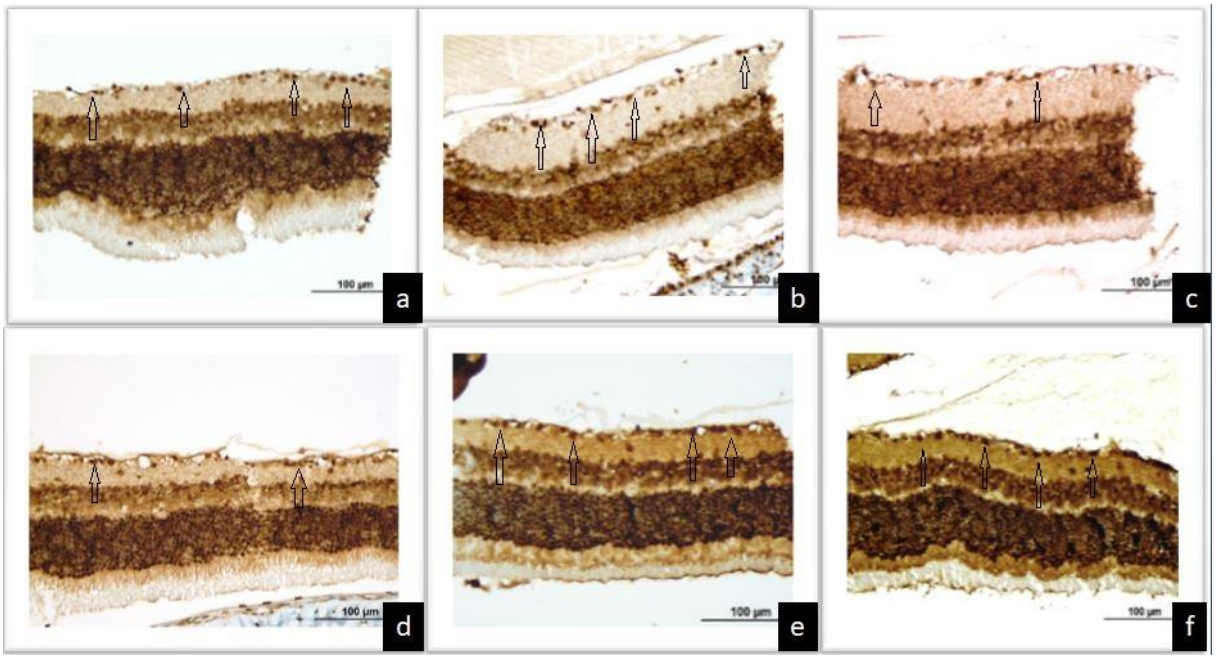

Figure 3. Representative images of Brn3a staining in retinal sections from different experimental groups. (a) Group I and (b) Group II exhibit increased ganglion cell staining, as indicated by the arrows. In contrast, (c) Group III and (d) Group IV show decreased ganglion cell staining, as marked by the arrows. Notably, (e) Group V and (f) Group VI demonstrate a significant increase in ganglion cell staining following treatment, as highlighted by the arrows.

MDA Levels

Mean MDA levels were 2.31 ± 0.31 for group 1, 2.34 ± 0.15 for group 2, 3.23 ± 0.85 for group 3, 2.24 ± 0.38 for oral TG, 2.27 ± 0.13 for drop TG, and 2.54 ± 0.29 for drop BT. MDA levels in group 3 were significantly higher than in group 1 ($p < 0.001$). Compared to group 3, MDA levels were significantly lower in the oral TG, drop TG, and drop BT groups ($p_{oralTG} = 0.038$, $p_{dropTG} = 0.033$, $p_{dropBT} = 0.019$)(Table 2).

	Group 1	**Group 2**	**Group 3**	**Oral TG**	**Drop TG**	**Drop BT**
MDA(nmol/mg)	2,31± 0.31	2,34± 0.15[b,c]	3,23± 0.85[a]	2,24± 0.38[b,c]	2,27± 0.13[b,c]	2,54± 0.29[b,c]
SOD(ng/L)	31,51± 8.04	37,36±8.38[b,c]	14,6± 4.01[a]	34,73±5.16[b,c]	38,46±3.60[b,c]	35,98±4.89[b,c]

Table 2. MDA and SOD levels (a: $p<0.05$ compared to the group 1; b: $p>0.05$ compared to the group 1; c: $p<0.05$ compared with group 3; d: $p>0.05$ compared with group 3).

SOD Levels

Mean SOD levels were 31.51 ± 8.04 for group 1, 37.36 ± 8.38 for group 2, 14.6 ± 4.01 for group 3, 34.73 ± 5.16 for oral TG, 38.46 ± 3.60 for drop TG, and 35.98 ± 4.89 for drop BT. We observed no significant difference between groups 1 and 2 ($p > 0.05$). SOD levels in group 3 were significantly lower than in group 1 ($p < 0.001$). The oral TG, drop TG, and drop BT groups had significantly higher SOD levels compared to group 3 ($p < 0.001$ for all) (Table 2).

iNOs Protein Expression Levels

iNOs protein expression levels increased by approximately 32% in group 3 compared to the control group ($p < 0.005$).We observed that the levels of iNOs protein expression were lower in the oral TG group (about 15%), the drop TG group (about 21%), and the drop BT group (about 20%). The drop TG and drop BT groups had significant drops (pdropTG = 0.037 and pdropBT = 0.04), but the oral TG group did not change significantly ($p > 0.05$).

4. DISCUSSION

Glaucoma is marked by the death of RGCs mainly through apoptosis, which is caused by things like high IOP, immune system reactions, and genetic interactions. Research highlights the involvement of various pathways, including oxidative stress, matrix metalloproteinases, and cytokine signaling, which underscore the disease's complexity (17-19). Understanding these multifactorial mechanisms is crucial for developing targeted treatments aimed at mitigating the progression of glaucoma. Neuroprotection protects susceptible or dying neurons, even if the host factor remains. Calcium channel blockers, NMDA antagonists, NOS inhibitors, neurotrophins, and antioxidants have neuroprotective activity in the eye (20). Rats with NMDA-induced retinal apoptosis tested TG for its neuroprotection and antioxidant properties. Compared to positive control groups, reduced TG dramatically decreased iNOS, MDA, caspase-3, and raised SOD.

Research has consistently shown that elevated IOP plays a significant role in initiating RGC apoptosis in glaucoma (21). In glaucoma, oxidative stress, inflammation, and changes in the extracellular matrix have also been linked to RGC apoptosis (22). Researchers have also identified mechanisms like autophagy, ischemia, and glutamate excitotoxicity as contributors to RGC death in glaucoma (23). Cells programmed for apoptosis can die in two main ways: intrinsically and extrinsically. Things inside the cell, such as free oxygen radicals and NMDA-mediated excitotoxicity, cause intrinsic death. Extrinsic death is caused by death receptors like TNF-α, FasL, and TLR. Both pathways ultimately activate caspase-3, leading to apoptosis (24). Caspase-3 serves as a crucial apoptosis indicator. Researchers are trying caspase-3 inhibitors as a new treatment option to increase RGC survival, as an increase in caspase-3 levels indicates increased apoptosis (25). A previous study examined the effectiveness of TG on hydrogen peroxide-induced apoptosis in cardiocytes. TG controls the apoptotic genes caspase 3 and caspase 9, as well as the antiapoptotic genes Bcl-2 and Bcl-xl (26). Our results of Caspase 3 immunohistochemistry analysis show that treatments in groups 5 and 6 significantly lower apoptosis compared to the NMDA-induced damage seen in Group 3. This demonstrates their

potential in mitigating retinal damage and underscores the effectiveness of these therapies in preserving retinal ganglion cell health.

Oxidative stress is a key part of how glaucoma develops. Reactive oxygen species (ROS) damage important eye structures like the cornea, trabecular meshwork, and optic nerve, which speeds up the disease's progression (27). This oxidative damage, compounded by aging, leads to neurodegenerative changes in RGCs, contributing to nerve degeneration (28). Studies show that glaucoma patients deplete antioxidants like glutathione, underscoring the importance of oxidative stress in the disease's chronic conditions (22). Further, oxidative stress-induced DNA damage in the trabecular meshwork can cause cell depletion, exacerbating glaucoma's pathology (29). Our study finds that TG treatment (oral TG, drop TG) effectively improves oxidative stress markers in neurodegenerative conditions such as glaucoma. This aligns with findings that nitric oxide (NO), which promotes apoptosis and free radical production, exacerbates neurodegeneration. TG's ability to reduce MDA levels and enhance SOD activity underscores its potential for mitigating oxidative damage, suggesting its therapeutic value in managing diseases influenced by oxidative stress.

Researchers have studied trigonelline, a notable alkaloid present in coffee, for its potential to modulate neuroinflammatory pathways in various neurological disorders, including Alzheimer's, Parkinson's, and multiple sclerosis. TG has been shown to lower MDA levels and acetylcholine esterase activity in the hippocampus of rats while increasing antioxidants like SOD, glutathione, and catalase. Additionally, it has been shown to lower levels of pro-inflammatory markers such as Nuclear Factor kappa B (NF-κB), TLR4, and TNF-α. This reinforces its neuroprotective properties, particularly in Parkinson's disease models, by increasing antioxidant enzymes and decreasing MDA (30,12). However, despite these promising results in general neurological models, our findings suggest that the neuroprotective effects of systemic TG administration do not translate similarly to retinal neuroprotection. This disparity underscores the importance of drug delivery methods for therapeutic effectiveness. In contrast, our results indicate that topical application of TG in the eye leads to lower apoptosis rates and better antioxidant profiles, suggesting that local administration might be more effective for retinal protection.

Studies have utilized TUNEL staining to examine cell proliferation and apoptosis in glaucoma (31,32). Scientists have used TUNEL staining to see how a treatment affects RGCs in mouse retinal tissues (33). TUNEL staining has also been very useful for finding cells that

have died in the RGC layer of different glaucoma models (34). In our study on glaucoma, we used TUNEL staining, which is a common way to check for apoptosis, to find the apoptotic index in different treatment groups. This index shows the percentage of apoptotic cells in the retinal tissues. As others have done, we looked at where TUNEL+ and Brn3a+ cells were together to find apoptosis in RGCs (35-37). Research has demonstrated that the Brn3a family is crucial for the differentiation, survival, and axonal elongation of RGCs during their development. Brn3a specifically stains RGCs (38). In our study, we observed a correlation between the number of TUNEL+ cells and Brn3a+ cells. Similar to previous studies, our findings revealed a significant decrease in the apoptotic index in the groups treated with Drop TG and BT, indicating their potential to safeguard neurons.

BT, an alpha-2 adrenergic receptor agonist, is a valuable treatment option for glaucoma and ocular hypertension due to its ability to lower IOP and its neuroprotective effects. It is believed to guard against RGC death and optic nerve damage by altering glutamate transporters and NMDA receptors, thereby halting excitotoxicity (39). In our study, using BT supports its neuroprotective effects seen in experimental models. Similarly, treatments with TG have shown similar neuroprotective outcomes, showing that both agents may be useful for keeping the retina and optic nerve healthy (40).

While this study offers valuable insights into the neuroprotective effects of TG and BT in glaucoma treatment, it should acknowledge several limitations. Firstly, the results from our animal models may not fully translate to human clinical outcomes due to physiological differences between species. We also saw important neuroprotective effects when TG was applied topically. However, more research is needed to find out how well and safely these treatments work in the long term on humans. Moreover, this study did not address dose optimization for topical TG, which is crucial for understanding the dose-response relationship and ensuring maximum therapeutic efficacy in future clinical applications.

5. CONCLUSION

Our study's findings highlight the potential of TG and BT as effective neuroprotective agents in glaucoma treatment, particularly in reducing apoptosis and enhancing antioxidant defenses in the retina. TG, applied topically, showed promising results in mitigating retinal damage similar to BT, suggesting its utility in eye disease treatments where oxidative stress plays a significant role. Future studies should focus on clinical trials to validate these findings and

potentially incorporate these treatments into standard glaucoma management protocols, offering hope for preserving vision in patients with this challenging condition.

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