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THERAPEUTIC EFFICIENCY OF RANIBIZUMAB AND 4-METHYL CATECHOL ON THE PROGRESSION OF STREPTOZOTOCIN-INDUCED DIABETIC RETINOPATHY IN RATS: INVOLVING VASCULAR ENDOTHELIAL GROWTH FACTOR-A AND NERVE GROWTH FACTOR

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Purpose: *Diabetic retinopathy (DR) is a chronic microvascular diabetic complication leading to visual loss. Ranibizumab (RBZ) is a monoclonal antibody inhibiting VEGF-A, approved for DR treatment. 4-Methyl catechol (4MC) is a potent NGF stimulant & possesses a neuroprotective effect in neuronal diseases. The aim of this study was to evaluate the role of 4MC in comparison to RBZ in the progression of streptozotocin (STZ) induced-diabetic retinopathy in albino rats.* ***Methods:*** *This experiment was performed on 40 albino rats divided equally into: (normal control, untreated DR, RBZ treated intravitreally & 4MC treated intravitreally).* ***Results:*** *The untreated DR group revealed a significant elevation of fasting serum glucose, VEGF-A, histopathological score & IHC score of Tie-2, and a significant decrease in NGF, and IHC of Bcl-2 versus the normal control group. Treatment with either RBZ or 4MC produced significant improvement in the forementioned parameters when compared to the untreated DR group.* ***Conclusion:*** *RBZ and 4MC are effective in the progression of STZ-induced DR via inhibition of VEGF-A and stimulation of NGF respectively*

Keywords: *Diabetic retinopathy, Streptozotocin, Ranibizumab, 4-Methyl catechol*

INTRODUCTION

Diabetes mellitus (DM) causes hyperglycemia, insulin dysregulation, and severe chronic consequences. More than 537 million people globally had DM in 2021, and this number is rising[1.] Diabetic retinopathy (DR) is the primary etiology of recently identified instances of visual impairment among adults. About 2.6 million individuals experienced visual impairment as a result of DR, and this number is expected to increase upto 4.5 million by the year 2045[2]. DR has variable stages that range from mild asymptomatic non-proliferative diabetic retinopathy to proliferative diabetic retinopathy[3].

The pathogenesis of DR is multifactorial. Poorly controlled hyperglycemia accounts for oxidative damage and neurodegeneration. However, the pathological picture of DR has been found in the retinas of rats even after months of normoglycemia achieved by insulin administration[4].

In experimental models of DR and human patients, the hallmarks of diabetes-induced neuroglial degeneration, including reactive gliosis and neural-cell apoptosis, have been reported to occur before overt microangiopathy[5]. NGF and other neurotrophins are crucial to DR pathogenesis. It was observed that neurotrophins expression increases because of oxidative stress induced by hyperglycemia leading to the protection of

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the neural retina and prolongation of its survival [6].

Tunica interna endothelial cell kinase-2 (Tie-2) activation by angiopoietin-1 is essential to preserve endothelial integrity, but stimulation of the same receptor by angiopoietin-2 causes disruption and inflammation of the endothelium lining blood vessels in the retina, which is responsible for the occurrence of cessation of retinal blood supply and new vessels formation [7].

Vascular endothelial growth factor-A (VEGF-A) is secreted from endothelial cells in response to hypoxia, stimulating both VEGFR-1 & VEGFR-2 leading to retinal inflammation & angiogenesis[8].

Bcl-2 is an antiapoptotic protein that inhibits necrotic cell death. Overexpression of Bcl-2 increased cellular resistance to hypoxia, excitotoxicity, calcium overload & axotomy leading to protection against cell death in early retinopathy[9].

The management of microvascular issues in DR involves many treatment modalities, such as intravitreal pharmacologic medicines, laser photocoagulation, and vitreous surgery. Intravitreal anti-VEGF medications are the cornerstone treatment for early and advanced DR. While traditional laser therapy simply stabilizes visual acuity, anti-VEGF therapy can improve vision with fewer side effects [10]. Although intravitreal steroid injection can also be effective in diabetic macular edema (DME) treatment, its application is restricted due to its ocular unfavorable effects [11].

Ranibizumab (RBZ) is a biopharmaceutical agent that belongs to the class of recombinant humanized monoclonal antibodies. Its mechanism of action involves the specific inhibition of VEGF-A, preventing it from binding to its receptors. It also proved to prevent apoptosis in age-related macular degeneration & endometriosis via increasing Bcl-2 expression [12]. The US-FDA recently approved it for neovascular acute macular degeneration (AMD), DR, and DME [13] with a 78% success rate [14].

Therefore, it is important to find and investigate new drugs targeting other factors like the neurotrophic factors to achieve better early curative results.

Chemical flavonoid compound 4-Methylcatechol (4MC) is a metabolite of polyphenols that was investigated and reported as a strong stimulator of endogenous NGF synthesis both in vitro and in vivo. It showed also antiplatelet, cardioprotective, antidepressant, and anticancer effects [15]. 4MC was shown to ameliorate various neurological diseases e.g. diabetic neuropathy, depression via induction of BDNF. It also prevents STZ-induced acute renal injury through modulating NGF/TrkA and ROS -related Akt/GSK3β/β-catenin pathways [16].

MATERIALS AND METHODS

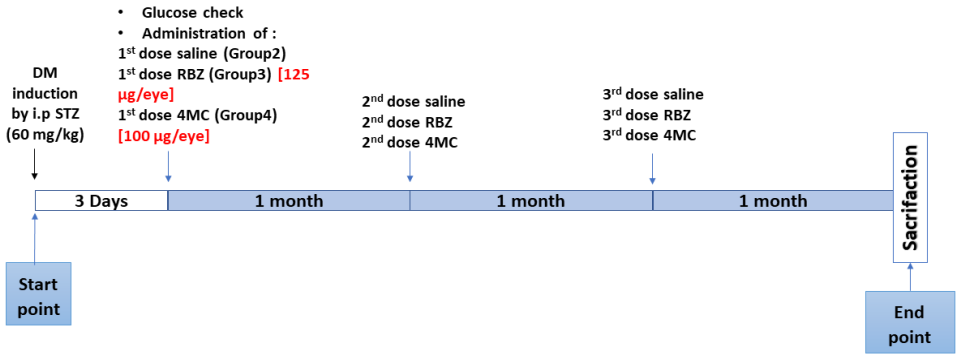
All protocols involving the care and use of animals in experiments followed the NIH's Guide for Care and Use of Laboratory Animals, and were approved by the "Research Ethics Committee, REC" at the Faculty of Medicine, Tanta University, Egypt (Approval number. 34439/2/21).

This study was carried out on 40 male Sprague Dawley albino rats, aged 7-8 weeks, weighing (150-200gm). The animals were housed in wire mesh cages in the animal house of the faculty of medicine, Tanta university and fed a standard animal diet with free access to water. The standard experimental conditions included 12 h-light/12 h-dark cycles and 23 ◦C–26 ◦C temperature. The rats were allowed a 10-day acclimatization span prior to the start of the experiment. Animals were divided into 4 groups (10 rats each) as follows **(Table 1)**:

- **Group 1**: normal control group received single intraperitoneal (i.p) injection of citrate buffer,
- **Group 2**: DR group, received vehicle of isotonic saline monthly intravitreally,
- **Group 3**: DR rats treated with RBZ (5 mg/0.5 ml purchased from Novartis Pharma AG, USA) intravitreally in a dose of 125 µg/eye/month **[17]**,
- **Group 4**: DR rats treated with 4MC (purchased from Sigma Aldrich, USA, (catalog No: 452-86-8), dissolved in isotonic saline to a final concentration of 10 mg/ml) intravitreally in a dose of 100 µg/eye/month **[18]**. All treatment protocol was applied on the 3rd day after induction of T1DM for 3 months **(Fig.1).**

Table 1: Treatment protocol of all groups.

Group	n	Treatment
Group 1	10	Normal control group received single (i.p) injection of 0.5 ml citrate buffer
Group 2	10	DR rats, received a vehicle of 10 µl isotonic saline monthly intravitreally for 3 months.
Group 3	10	DR rats treated with RBZ (5 mg/0.5 ml) intravitreally in a dose of 125 µg/eye/month for 3 months.
Group 4	10	DR rats treated with 4MC, dissolved in isotonic saline to a final concentration of 10 mg/ml) intravitreally in a dose of 100 µg/eye/month for 3 months.



STZ: streptozotocin, RBZ: ranibizumab, 4MC: 4-methyl catechol

Fig. 1: Summary diagram of the course of the experiment.

Induction of T1DM

STZ (purchased from Sigma Aldrich, USA (catalog No: 18883-66-4)) was injected once (i.p). in a dose of 60 mg/kg to induce T1DM. Glucose 5% solution was added on the day of induction to the drink of the rats to avoid hypoglycemia in the first 24 hours. The fasting blood glucose level was checked 3 days later using a blood sample from the tail where rats with glucose levels ≥ 200 mg/dl were considered diabetic [20].

Intravitreal drug administration protocol

The intravitreal injection was carried out following the technique described previously by Said et al (2017)[17] and Filek et al (2019)[22] under strict sterilization conditions as follows: Rat was anesthetized using (i.p.) injection of ketamine hydrochloride [(KETAM, Egyptian International pharmaceutical industries company E.I.P.I.CO) (40 mg/kg), and xylazine hydrochloride (5 mg/ml)], Then, we anesthetized both eyes with topical eye drops of benoxinate ophthalmic solution (0.4%) obtained from Egyptian Pharmaceutical Industries Co (E.P.I.CO), Both pupils were dilated via tropicamide eye drops 1% obtained from Alcon pharmaceutical company, Using a 30-gauge Hamilton's syringe, we injected the upper nasal part of the sclera 1.5 mm behind the limbus using a magnifying lens (X2.5), the needle tip was observed during the procedure to avoid retinal injury and remained in the eye for 3-4 seconds to avoid reflux.

The surviving rats were anesthetized with i.p. pentobarbital (50 mg/kg) at the end of the experiment, and blood samples were collected via intracardiac puncture in plain tubes to be centrifuged at 4000 rpm for 15 minutes, and serum was separated to assay fasting glucose levels spectrophotometrically using a kit from Biodiagnostic Company (catalog No. GL 1320) according to the method described by Trinder (1969)[23], then both eyes were excised. The right eye was dissected, and the retina was extracted and processed for histopathological and transmission electron microscope (TEM)

examination and immunohistochemical (IHC) assay of Bcl2, Tie-2.

Biochemical ELISA measurement of VEGF-A & NGF

Tissue samples of the left eye were weighed. Add phosphate buffered saline (pH 7.4) then homogenized by Teflon homogenizer, centrifuged for 20-min at 3000 r.p.m. The supernatant was collected and rapidly frozen at -80 ℃, maintaining samples at 2-8℃ after melting, to be used for assay of VEGF-A (catalog No.: 201-11-5123) and NGF (catalog No.: 201-11-0540) by rat ELISA kits (SunRed Biotechnology Company, China,) following the manufacturer protocol.

Light microscopic examination & scoring of the retinal tissue

The right eye globe samples were immediately fixed in 10% formalin. Then, 5 µm paraffin sections were prepared and stained with hematoxylin and eosin (H&E). A total of six sections were extracted from each sample, and subsequently, five high-power visual fields (×400) were captured from each section to be examined by light microscope for histopathological changes and scoring, according to the following criteria: normal (0 points); rod cell, and cone cell destruction (1–2 points); outer nuclear layer failure (3–4 points); plexiform layer corruption (5–6 points); and ganglion cell layer destruction (7 points) **[24].**

Transmission electron microscopic (TEM) examination of the retina

After right eye enucleation, the retinas were dissected and preserved in 2.5% glutaraldehyde at 4 ℃ for 2 h. Ultrathin 50–60 nm ultramicrotome sections were stained with 3% uranyl acetate-lead citrate and analyzed by TEM (×2500) **[25].**

IHC expression of Bcl-2 & Tie-2 in the retina

were detected using rabbit anti-human Bcl-2 monoclonal antibody (Clone EP36) purchased from MASTER DIAGNOSTICA, Granada, Spain and rabbit anti-human Tie-2 monoclonal antibody purchased from Bioassay technology laboratory company (code: BT-AP14960) Shanghai, China, respectively.

Interpretation of IHC score

The software (Image J version 1.2.4) (National Institute of Health, Bethesda, Maryland, USA) **[26]** was used to score Tie-2 & Bcl-2 expression by multiplying the color intensity x the percentage of positively stained cells[27].

Statistical analysis

Results were analyzed statistically using the software Statistical Package for the Social Science (SPSS) for Windows, version 23, (SPSS Inc., USA). The values were analyzed by the Shapiro-Wilk test for normality of distribution where the descriptive statistics of parametric values were presented as Mean ± Standard Deviation (S.D). The one-way ANOVA test was used to test the significance of the difference between more critical values corresponding to areas of 0.05 under the upper tail distribution. This was followed by post-hoc **Tukey's Test** to test the difference between each two means. The level of significance was established at values of $p< 0.05$.

RESULTS AND DISCUSSION

Results

The statistics of the results were summarized in **(Table.2)**.

Fasting serum glucose level

The untreated group showed a significant elevation of fasting serum glucose levels ($p<0.05$) in comparison to the normal control group while RBZ-treated and 4MC-treated groups showed a non-significant difference in fasting serum glucose levels in comparison to the untreated group **(Fig.2).**

RBZ reduced eye tissue VEGF-A level

The untreated group showed a significant elevation of eye tissue VEGF-A levels ($p<0.05$) in comparison to the normal control group while RBZ induced a significant decrease in VEGF-A levels ($p<0.05$) in comparison to the untreated group. However, 4MC significantly elevated VEGF-A levels in comparison to RBZ **(Fig. 3).**

Table 2: Summary of the experimental results. # significant versus group 1(p<0.05), * significant versus group 2 (p<0.05), $ significant versus group 3 (p<0.05).

Groups / Parameter	Group 1 (Normal control) (n=10)	Group 2 (Untreated DR) (n=10)	Group 3 (DR, RBZ treated) (n=10)	Group 4 (DR, 4MC treated) (n=10)	One-way ANOVA F value (P value)
Fasting serum glucose levels (mg/dl)	102.2± 9.86	484.7±71.29#	518.2±93.21	452± 84.91	62.44 (P<0.05)
Eye tissue VEGF-A levels (ng/L)	22.67±1.7	49.79±2.57#	38.6± 3.25*	45.57 ± 5.96 $	83.36 (P<0.05)
Eye tissue NGF levels (ng/ml)	10.53 ± 1.015	6.611±1.5#	7.877±0.786	11.38 ± 1.35 *$	29.47 (P<0.05)
H&E score	0.86±0.67	15.79±2.49#	8.12±1.13*	9 ± 3.54*	58.96 (P<0.05)
Bcl-2 IHC score.	0.584 ± 0.044	0.279±0.017#	0.404±0.011*	0.415± 0.057*	86.82 (P<0.05)
Tie-2 IHC score.	1.811 ± 0.08	5.45±1.001#	1.48±0.19*	0.97 ± 0.2*	140.2 (P<0.05)

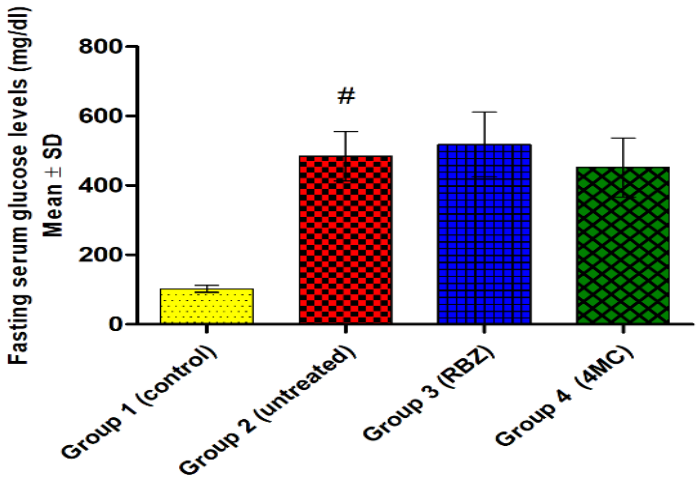


Fig. 2: The high fasting serum glucose in groups 2, 3 & 4 is due to the effect of STZ (Not related to RBZ or 4MC), # significant versus group 1 (p<0.05).

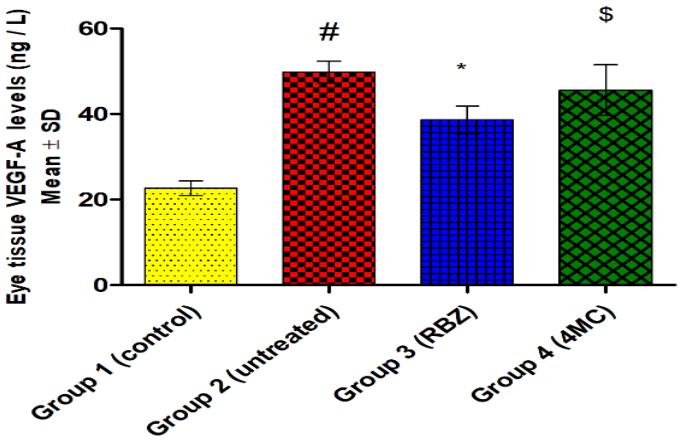


Fig. 3: Effect of RBZ & 4MC on eye tissue VEGF-A levels. # significant versus group 1(p<0.05), * significant versus group 2 (p<0.05), $ significant versus group 3 (p<0.05).

4MC enhanced eye tissue NGF level

4MC significantly increased eye tissue NGF levels in comparison to the untreated group & RBZ treated group **(Fig. 4).**

Light microscope histopathological (H&E) examination & scoring

Histopathologically RBZ & 4MC preserved the retinal layer structure in comparison to the untreated group. This was confirmed by the histopathological score as both RBZ and 4MC significantly lowered the score in comparison to the untreated group. However, there was no significant difference between RBZ & 4MC treated groups **(Fig. 5, Table 3)**.

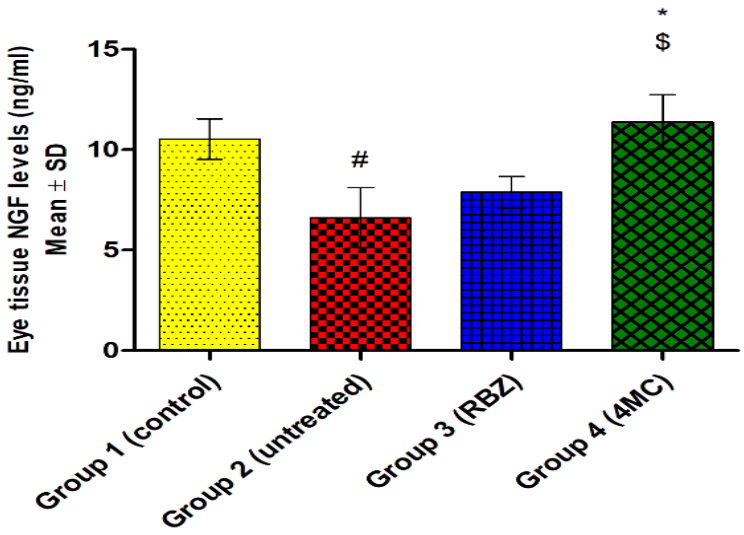


Fig. 4: 4MC induced an increase in eye tissue NGF level more than RBZ. # significant versus group 1($p<0.05$), * significant versus group 2 ($p<0.05$), $ significant versus group 3 ($p<0.05$).

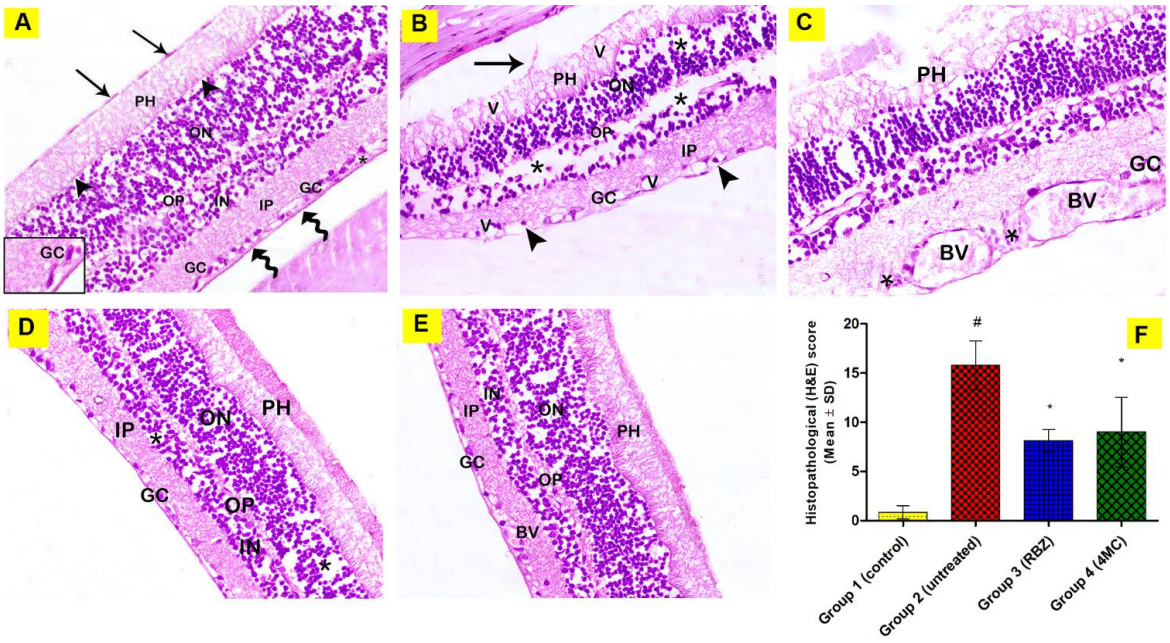


Fig. 5: Light microscopic (H&E) examination & scoring of the rat retinae (X400) F: Both RBZ & 4MC induced improvement of the histopathological score. # significant versus group 1($p<0.05$), * significant versus group 2 ($p<0.05$), $ significant versus group 3 ($p<0.05$).

Table 3: Light microscopic (H&E) findings in rat retinae.

Groups	H & E examination results
Group 1	All layers are normal.
Group 2	Degeneration of all layers, focal separation of RPE, leukostasis, dilated & congested blood vessels.
Group 3	Apparently preserved all retinal layers except few signs of degeneration in the inner & outer nuclear layers.
Group 4	Apparently preserved histological structure of all retinal layers except mildly dilated capillaries in the ganglion cell layer.

Transmission electron microscopic examination (TEM) (X2500)

Both RBZ & 4MC improved the TEM picture of retinal layers in comparison to the untreated group, but 4MC improved the inner segment of the photoreceptor layer & inner nuclear layer (INL) more than RBZ. **(Table 4).**

Immunohistochemical (IHC) examination & scoring of Bcl-2 (X400)

Immunohistochemically both RBZ & 4MC enhanced Bcl-2 expression in RPE, PH layer & GC layer in comparison to the untreated group. However, there was no significant difference in immunohistochemical scoring between both drugs **(Fig.6)**.

Immunohistochemical (IHC) examination & scoring of Tie-2 (X400)

Immunohistochemically both RBZ & 4MC enhanced Tie-2 expression in RPE, PH layer & GC layer in comparison to the untreated group. However, there was no significant difference in immunohistochemical scoring between both drugs **(Fig.7**).

Table 4: TEM results of all groups.

Group	Outer segment of photoreceptor layer	Inner segment of photoreceptor layer	Ganglion cell layer	Inner nuclear layer
Group 1	Normal	Normal	Normal	Normal
Group 2	Markedly disrupted membranous discs with wide intersegment spaces	Mitochondrial degeneration, with interrupted thin outer limiting membrane	Thickened basement membrane of endothelial lining capillaries, swollen endothelial cells with mitochondrial degeneration, some degenerated ganglion cells	Retinal neuron & Muller cell degeneration.
Group 3	Almost preserved discs	Almost preserved inner segment with normal mitochondria, some degenerated mitochondria were seen.	Almost preserved ganglion cell structure with apparently normal mitochondria, normal endothelial cells.	Nearly normal retinal neurons & Muller cells.
Group 4	Apparently normal discs with minimal disc interruption.	Apparently normal inner segment with few cytoplasmic vacuoles (degeneration marker)	Nearly normal ganglion cell with mildly dilated rER.	Normal Muller cells.

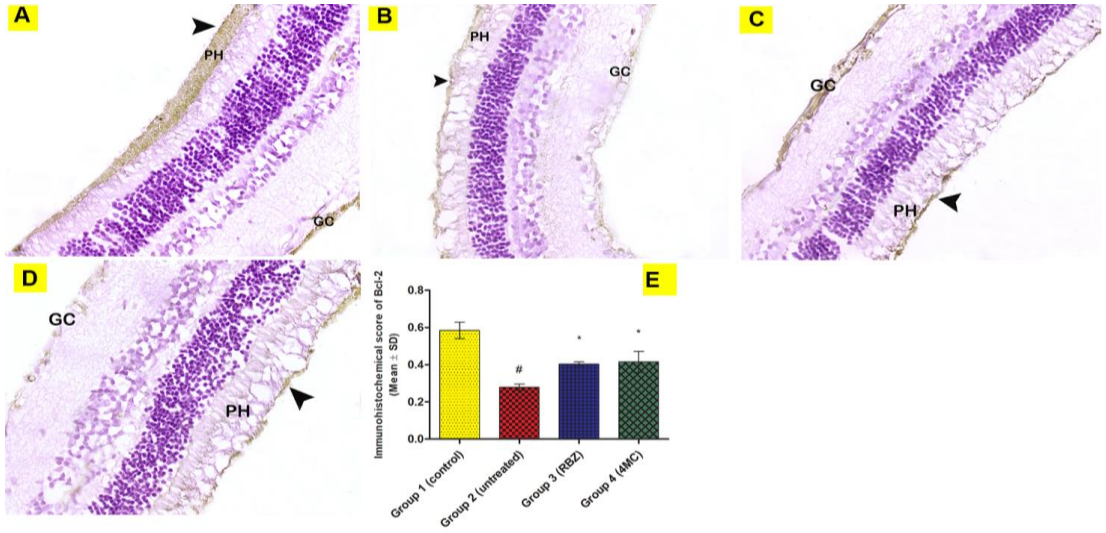


Fig. 6. A: IHC expression of Bcl-2 in group 1 (normal control) revealed a strong positive cytoplasmic immunostaining for Bcl-2 in the retinal pigmented epithelium (arrowhead), photoreceptors (PH) and ganglion cell layers (GC). **B:** IHC expression of Bcl-2 in group 2 (untreated DR) revealed a faint positive cytoplasmic immunostaining for Bcl-2 in the retinal pigmented epithelium (arrowhead), PH and GC. **C:** IHC expression of Bcl-2 in group 3 (DR, RBZ treated) revealed a strong positive cytoplasmic immunostaining for Bcl-2 in the retinal pigmented epithelium (RPE) (arrowhead), PH and GC. **D:** IHC expression of Bcl-2 in group 4 (DR, 4MC treated) revealed a moderate positive cytoplasmic immunostaining for Bcl-2 in the RPE (arrowhead), PH and GC. **E:** Both RBZ & 4MC induced improvement of IHC score of Bcl-2. # significant versus group 1($p<0.05$), * significant versus group 2 ($p<0.05$), $ significant versus group 3 ($p<0.05$).

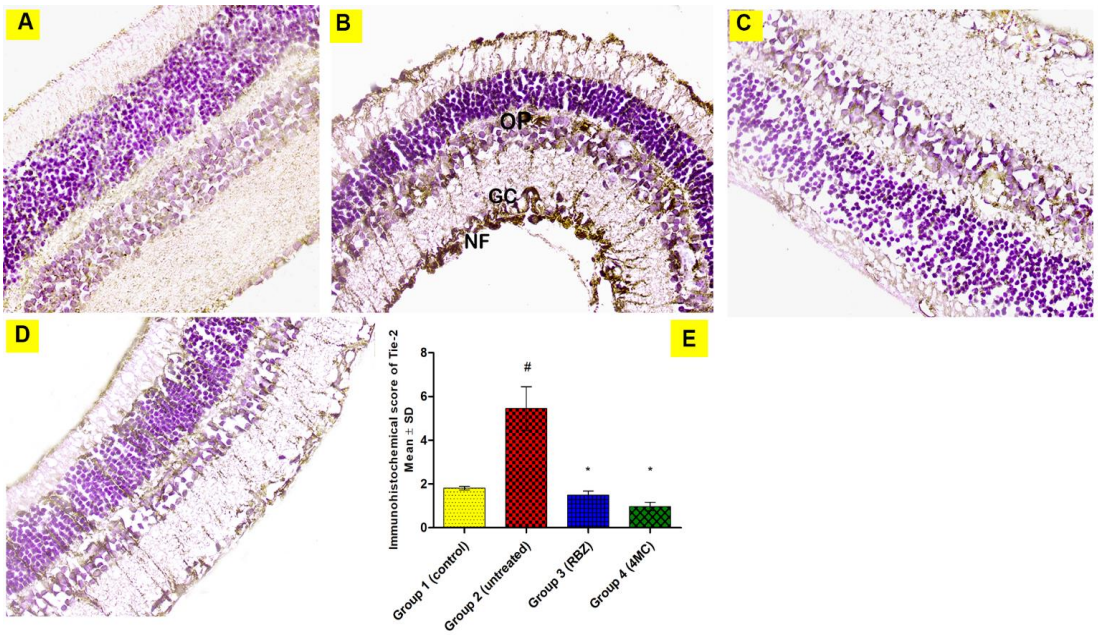


Fig. 7. A: IHC expression of Tie-2 in group 1 (normal control) showed faint positive immunostaining for Tie-2 in almost all retinal layers. **B:** IHC expression of Tie-2 in group 2 (untreated DR) showed an intense positive immunostaining for Tie-2 in retinal layers especially OPL, GC and nerve fiber layer (NF). **C:** IHC expression of Tie-2 in group 3 (DR, RBZ treated) showed mild positive immunostaining for Tie-2 in retinal layers. **D:** IHC expression of Tie-2 in group 4 (DR, 4MC treated) showed moderate positive immunostaining for Tie-2 in retinal layers. **E:** RBZ & 4MC induced normalization of IHC score of Tie-2. # significant versus group 1($p<0.05$), * significant versus group 2 ($p<0.05$), $ significant versus group 3 ($p<0.05$).

Discussion

DR is a highly widespread complication of diabetes and is considered a prominent cause of visual impairment globally [28]. The pathophysiology of DR is multifactorial including hyperglycemia which triggers oxidative stress, and retinal inflammation with early neuronal and subsequent vascular affection of the retina [29]. The pharmacological management of DR includes control of blood glucose, blood pressure and blood lipids. However, glycemic control of DM only cannot prevent the occurrence of DR but may help in slowing its progression. Currently, the FDA-approved pharmacotherapy directing DR includes Intravitreal injection of drugs: anti-VEGF (ranibizumab, aflibercept), corticosteroids (fluocinolone) and NSAID (ketorolac, diclofenac) [30]. In the untreated DR group, this single dose of STZ was sufficient to induce hyperglycemia that lasted for 3 months without any signs of recovery until the experiment was completed, manifested as a significant increase of serum glucose in the untreated DR group in comparison to normal control group.

Regarding the eye tissue VEGF levels, the untreated DR group revealed significantly increased retinal VEGF levels in comparison to the normal control group which could be attributed to hypoxia-inducible factors [31] and autocrine loop induced by oxidative stress [32]. RBZ significantly decreased retinal VEGF levels in comparison to the untreated DR group due to the anti-VEGF effect of RBZ which agreed with previous studies that found species-specific affinity of RBZ to VEGF-A in humans & rats, and it was effective in lowering retinal VEGF levels in the murine ischemic model & diabetic patients [33, 34]. Ang 1/Tie-2 interaction could induce vascular remodelling by facilitating tight endothelial junction unlike VEGF which increased vascular leakage [35].

Regarding eye tissue NGF levels, The neuronal deficit was confirmed in the untreated group by the significant decrease in retinal NGF versus the normal control group which occurred due to disrupted proNGF/NGF ratio with concurrent elevation of proNGF. Such deficit could be attributed to hyperglycemia that impaired proteolytic cleavage of proNGF to yield NGF, so the increasing proNGF led to activation of nuclear factor-kappa B (NF-κB) then production of other proinflammatory cytokines and activation of Ras homolog family member A/ Mitogen-activated protein kinases (RhoA/MAPK) pathway leading to neuronal death and also would activate c-Jun kinase (JNK) inducing endothelial apoptosis and formation of acellular capillaries [36, 37]. NGF could stimulate either TrkA inducing neuronal cell survival or p75 Neurotrophin receptor (NTR) inducing secretion of inflammatory mediators leading to neuronal and endothelial cell death. However, NGF binds preferentially to TrkA more than p75NTR [38]. 4MC significantly increased the retinal NGF levels versus the untreated DR group and RBZ denoting the NGF stimulant effect of 4MC. Previous studies confirmed the role of 4MC in neuroprotection via stimulation of NGF mRNA expression and documented the role of 4MC in skin regeneration via NGF stimulation in vivo and in vitro [39, 40]. NGF proportionally increased VEGF secretion in Müller cells via activation of extracellular signal-regulated kinase 1/2 (ERK 1/2) and PI3K/AKT pathway leading to Müller cells proliferation. This was a compensatory mechanism that helped to maintain the neuroprotective effect through the preservation of glial cells function which appeared early in DR but the late stages showed a marked decrease in retinal NGF levels [41, 42]. Also, Mantelli et al (2014)[43] stated that the early increase in endogenous NGF in the diabetic retina was a protective response for ganglion cells from degeneration, but this response was impaired at 11 weeks following DM induction resulting in significant ganglion cell degeneration that exhibited significant improvement by exogenous NGF administration but exacerbated by anti-NGF antibodies. However, his study showed that there was no alteration in VEGF levels in response to exogenous NGF or anti-NGF antibodies and remained high along the course of the disease.

According to the IHC scoring of Bcl-2, the present work showed a significant decrease in Bcl-2 immunohistochemical score in the untreated DR group in comparison to the normal control group which may be due to diabetes-induced oxidative stress that led to retinal cells apoptosis evidenced by the increase of caspase-3 expression [44]. Also, Wang et al (2019)[45] and Fu et al (2022)[46]

reported that the lowered levels of retinal tissue Bcl-2 by western blot analysis in diabetic rats led to apoptosis induction by Bax, p38 and p53 which were normally inhibited by Bcl-2. RBZ significantly increased the retinal Bcl-2 immunohistochemical score when compared to the untreated DR group. Abdel-Maged et al (2018)[47] documented the antiapoptotic effect of RBZ in rheumatoid arthritis as it lowered the Bax/Bcl2 ratio. An in vitro study conducted by De Cillà et al (2017)[48] on RPE showed the ability of RBZ to counteract the activation of apoptotic markers such as cytochrome c and caspase-9. Here 4MC significantly increased the immunohistochemical score of Bcl-2 versus the untreated DR group as 4MC stimulated NGF which increased Bcl-2/Bax ratio in epithelial ovarian cancer producing anti-apoptotic effect [49]. NGF regulated endothelial retinal cell survival via increasing Bcl-2 and decreasing Bax with enhanced ERK and AKT phosphorylation, and suppression of the proapoptotic activity of Bcl-2 homology-3 (BH3) [50, 51].

According to the IHC scoring of Tie-2, it was significantly increased in the untreated group versus the normal control group as Tie-2 levels were increased in the diabetic rats retinae which might be a compensatory trial to overcome the antagonistic effect of Ang2 which increased in diabetic retina due to hypoxia & hyperglycemia [52, 53]. However, Chatterjee et al (2020)[54] explained that Tie-2 expression was not related to hyperglycemia but rather attributed to nucleoside diphosphate kinase B (NDPK-B) deficiency-induced retinopathy which is a housekeeping enzyme that keeps blood vessels from leaking by controlling how caveolins and adhesion molecules are distributed among endothelial cells. However, these findings were not conclusive, and it was a matter of controversy. On the contrary, Park et al (2021)[55] stated that there was no significant difference in Tie-2 expression between diabetic and non-diabetic mice. RBZ induced a significant decrease in Tie-2 immunohistochemical score in comparison to the untreated DR group because RBZ decreased both mRNA and protein levels of Tie-2 in oxygen-induced retinopathy [56]. However, another recent study by Koh et al (2022)[57] stated that although VEGF-A inhibition counteracted the angiogenic process, Tie-2 inhibition that occurred with anti-VEGF drugs like RBZ could induce angiogenesis in the long run, so adjuvant treatment to anti-VEGF drugs with Tie-2 activating action e.g. AKB-9778 or Ang2 inhibiting action e.g. faricimab was much preferred than monotherapy with RBZ. In addition, 4MC induced a significant decrease in the immunohistochemical score of Tie-2 in comparison to the untreated DR group as 4MC was a potent BDNF stimulant which in early stages of DR could downregulate both VEGF and Tie-2 in optimum concentration [58]. To sum up, RBZ was superior on 4MC in lowering VEGF-A levels, while 4MC was superior on RBZ in increasing NGF levels. However, both RBZ and 4MC showed the same effect on Bcl-2 and Tie-2.

Conclusion

These findings suggest that RBZ was superior to 4MC in lowering VEGF-A levels, while 4MC was superior to RBZ & 4MC in increasing NGF levels. However, both RBZ & 4MC showed the same effect on Bcl-2 & Tie-2. So, RBZ could be recommended for vascular stages while 4MC could be recommended for early neuronal phase. Both RBZ & 4MC revealed promising effects in the prevention of STZ induced-DR. Further clinical studies could be recommended to verify these results.

Declarations

Ethical approval

All protocols involving the care and use of animals in experiments followed the NIH's Guide for Care and Use of Laboratory Animals, and were approved by the "Research Ethics Committee, REC" at the Faculty of Medicine, Tanta University, Egypt (Approval number. 34439/2/21)

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الكفاءة العلاجية للرانيبيزوماب و ٤-ميثيل كاتيكول في تطور اعتلال الشبكية السكري المستحث بواسطة الستربتوزوتوسين في الجرذان: إشراك عامل نمو بطانة الأوعية الدموية-أ وعامل النمو العصبي

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إن الاعتلال الشبكى السكرى هو مشكلة صحية شائعة، وهو مرض يستمر مدى الحياة ويؤثر على كفاءة العيش. السبب الدقيق لهذا المرض غير مفهوم تماما، فهو يشمل تفاعلات بين تلف الاوعية الدموية الدقيقة واضطرابات التمثيل الغذائي والالتهاب والاجهاد التأكسدى والنواتج النهائية للتسكر ونقص عوامل النمو. هناك العديد من العلاجات الطبية ولكن لها الكثير من العيوب. ان عقار الرانيبيزوماب هو جسم مضاد احادى النسيلة وهو مضاد لعامل نموالاوعية الدموية وهو دواء معتمد لعلاج الاعتلال الشبكى السكرى. ٤-ميثيل كاتيكول هو محفز قوى لعامل النمو العصبي وله تأثير وقائى للاعصاب فى مختلف الامراض العصبية.

الهدف من هذه الدراسة: هوتقييم ومقارنة الدور الوقائي المحتمل لكل من رانيبيزوماب و ٤-ميثيل كاتيكول فى تطور الاعتلال الشبكى السكرى المستحث بواسطة الستربتوزوتوسين في الجرذان البيضاء.

الطرق: أجريت الدراسة علي ٤٠ جرذ أبيض مقسمين إلي ٤ مجموعات متساوية كالأتي:

- مجموعة(١):المجموعة العادية الضابطة تم حقنها بجرعة واحدة من محلول السترات المتعادل داخل التجويف البريتونى.
- مجموعة (٢):مجموعة مصابة بالاعتلال الشبكى السكرى غير معالجة،و تم اعطاؤها جرعة واحدة من الستربتوزوتوسين (٦٠ مجم/كجم) داخل التجويف الريتونى ، وحقن محلول ملح متعادل داخل الجسم الزجاجى.
- مجموعة (٣): مجموعة مصابة بالاعتلال الشبكى السكرى تم حقنها برانيبيزوماب (١٢٥ ميكروجم / عين / شهر) داخل الجسم الزجاجى.
- مجموعة (٤): مجموعة مصابة بالاعتلال الشبكى السكرى تم حقنها ب ٤-ميثيل كاتيكول (١٠٠ ميكروجم / عين / شهر) داخل الجسم الزجاجى.

تم جمع عينات الدم لقياس نسبة الجلوكوز الصائم وتم ذبح جميع الجرذان وتشريح كرات العين ومعالجتها لقياس عامل نمو الاوعية الدموية وعامل النمو العصبي وكذلك لعمل الفحص النسيجى المرضى والميكروسكوب الالكترونى و قياس درجة التغير الكيميائي المناعي لكل من بي سي ال-٢ و تاى-٢.

النتائج: أظهرت المجموعة المصابة بالاعتلال الشبكى السكرى الغير معالجة زيادةذات دلالة احصائية في مستوى الجلوكوز الصائم وعامل نمو الاوعية الدموية ومقياس فحص الانسجة المرضى و درجة التغير الكيميائي المناعي لتاى-٢ ، كما اظهرت انخفاضا ذو دلالة احصائية فى عامل النمو العصبي ومقياس الكيمياء المناعية لبي سي إل-٢ مقارنة بالمجموعة العادية الضابطة. اظهرت الدراسة الحالية ايضا ان العلاج باستخدام رانيبيزوماب و ٤-ميثيل كاتيكول أدى الى تحسن كبير فى معظم المقاييس مقارنة بالمجموعة المصابة بالاعتلال الشبكى السكرى الغير معالجة، ومن المثير للاهتمام ان ٤-ميثيل كاتيكول تسبب فى زيادة ملحوظة لعامل النمو العصبي مقارنة برانيبيزوماب.

الاستنتاج: وتشيرهذه النتائج إلى أن رانيبيزوماب يوصي به فى المراحل المتعلقة بالأوعية الدموية بينما يوصي باستخدام ٤-ميثيل كاتيكول فى المراحل العصبية المبكرة. حيث أن كلا من رانيبيزوماب و ٤-ميثيل كاتيكول أعطى نتائج واعدة فى الوقاية من الاعتلال الشبكي السكري المستحث بواسطة ستربتوزوتوسين .