Egyptian Journal of Anatomy, Jan. 2017; 40(1):18-32

Original Article

The Possible Neuroprotective Role of Taurine on the Rat's Retinal Ganglion Cells against Glutamate - induced Toxicity

Kariman M. El-Gohari, Ibtisam A. Bahei-Eldin, Eman K. Habib, Shereen A. Saad, Hagar Y. Rady, Azza M. Said

Anatomy and Embryology Department and Ophthalmology Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt

ABSTRACT

Purpose: Glutamate is found in a wide variety of foods, as it has been shown to have a flavor-enhancing effect. It is added to foods – either as the purified monosodium salt or as hydrolyzed protein. As Glutamate is one of contributors to retinal ganglion cells (RGCs) degeneration. Therefore the aim of the present study was to investigate the possible neuroprotective role of taurine on the rat's RGCs against glutamate-induced toxicity using histological and immunohistochemical techniques.

Material and Methods: Twenty four adult Sprague Dawley male albino rats were divided into four groups. Control group: six rats were divided into two subgroups; Subgroup 1a included both eyes of three rats (6 eyes) that were left without any intervention. Subgroup 1b right eye of six rats (6 eyes) was injected intravitreally with 0.1 ml of balanced salt solution; 0.9% NaCl (BSS). Glutamate group: right eyes of 6 rats (6 eyes) were injected intravitreally with 0.1 ml of a single dose of monosodium glutamate (40 nmol/ml). Taurine group: three rats (6 eyes) received intraperitoneal injection of a single dose of taurine (25 mg / kg) B.W., which was dissolved in 1 ml of BSS and injected intraperitoneally. Combined Glutamate and taurine group: right eyes of 6 rats (6 eyes) were injected intravitreally with 0.1 ml of a single dose of monosodium glutamate (40 nmol/ml) and at the same time, each rat received a single dose of taurine intraperitoneally as in taurine group. Three days after injection, animals were sacrificed and eye balls were enucleated and processed for histological and immunohistochemical staining.

Results: Extensive damage and disruption of the structure of the retina following glutamate intravitreal injection was found. The photoreceptor layer showed marked irregular appearance, vacuole formation, focal loss and wide retinal blood vessel was seen within the GCL. Complete absence of ganglion cells with the presence of small dark glial cells within the GCL were noticed. There was a statistically significant decrease in the mean total retinal thickness. Also, there was decrease in the thickness of outer nuclear layer (ONL) and inner nuclear layer (INL). In addition to decrease in ganglion cell count. A significant improvement of this picture was observed in taurine and combined groups (*P*-values were < 0.001). In Taurine group preservation of normal architecture of all retinal layers was observed with presence of multiple blood vessels in some retinal layers. A statistically significant increase in glial fibrillary acidic protein and synaptophysin immunostaining was seen in most retinal layers in glutamate group compared to no or weak staining in the other groups (P-values < 0.001) however, negative or faint vascular endothelial growth factor and caspase-3 immunostaining was detected in all animal groups.

Conclusion: Taurine is protective to the retina against glutamate excitotoxicity and could have clinical implications in protecting the ganglion cells in several ophthalmic diseases as glaucoma and diabetic retinopathy.

Key Words: Glutamate, neuroprotection, retinal toxicity, taurine.

Corresponding Author: Eman Kamal Mohammed Habib, **E-mail:** emanhabib75@gmail.com, **Tel:** + 20 100 18 40 449

Personal non-commercial use only. EJA copyright © 2017. All rights reserved

INTRODUCTION

Glutamate is found in a wide variety of foods, and in its free form has been shown to have a flavor-enhancing effect. So it is often deliberately added to foods – either as the purified monosodium salt or as hydrolyzed protein (*Babai et al., 2005*).

The retina is the sensory neural layer of the eye. It is the most complex structure and should be considered as a special area of the brain. Retinal Ganglion cells (RGCs) are the final common pathway neurons of the retina and therefore are of critical importance to the visual system (*Standring, 2008*). Glutamate is the major excitatory neurotransmitter conducting visual signals within the retina. It is highly concentrated in the photoreceptors, bipolar cells and ganglion cells. However, if excessive amounts of glutamate are released or if glutamate clearance is insufficient, neuronal death can result in a process known as "excitotoxicity" (*Ishikawa, 2013*).

Glutamate-excitotoxicity has been proposed to be an important contributor to the death of (RGCs) in glaucoma, diabetic retinopathy and ischemia induced by retinal or choroidal vessels occlusion (*Hernandez & Simo, 2012*). These pathological conditions can be mimicked by experimentally elevating extracellular glutamate concentration or applying its analogues or sodium salt monosodium glutamate (*Dénes et al., 2011*). Glutamate-induced neuronal death is initiated by increase of intracellular free calcium and sodium level followed by activation of catabolic enzymes such as proteases, phospholipases, endonucleases, protein–kinase and lipid–kinase cascades. Energy compromise and formation of reactive oxygen species (ROS) lead to cell death (*Leon et al.,2009*).

Taurine (2-aminoethanesulfonic acid) is the most abundant free amino acid in the retina and is considered as a neurotransmitter. It serves in maintaining the structural integrity of the cell membranes of the RGCs regulating calcium binding and transport (Wu & Prentice, 2010). Therefore, the potential neuroprotective functions of endogenous molecules such as taurine are being considered (*Chen et al., 2009*). It has been successfully applied for the treatment of neurodegenerative diseases (*Buddhala et al., 2012*).

The aim of the present study was to investigate the possible neuroprotective role of taurine on the rat's RGCs against glutamate-induced toxicity using histological and immunohistochemical techniques.

MATERIAL AND METHODS

Study design

Twenty- four Sprague Dawley adult male albino rats weighing 180 - 200 gm were obtained from the animal house of the Medical Research Centre, Faculty of Medicine, Ain Shams University. All experiments were carried out in accordance with the Guide of the Committee of Animal Research Ethics (CARE)- Faculty of Medicine - Ain Shams University. All animals were divided into four groups as follows:

• Control group: six rats were divided into two subgroups; Subgroup 1a included both eyes of three rats (6 eyes) that were left without any intervention. Subgroup 1b right eye of six rats (6 eyes) was injected intravitreally with 0.1 ml of balanced salt solution; 0.9% NaCl (BSS) (*Hong-xia et al., 2005*).

• Glutamate group: right eyes of 6 rats (6 eyes) were injected intravitreally with 0.1 ml of a single dose of monosodium glutamate which was provided in a powder form (Al- Gomhuria chemical company, Cairo, Egypt). A single dose of 40 nmol glutamate was dissolved in 1 ml of BSS (*Hong-xia et al., 2005*).

• Taurine group: three rats (6 eyes) received intraperitoneal injection of a single dose of taurine. Taurine was provided in a powder form (Sigma, St Louis, Missouri, USA). A single dose of taurine (25 mg / kg) B.W. which was dissolved in 1 ml of BSS and injected intraperitoneally (*Hong-xia et al., 2005*).

• Combined Glutamate and taurine group: right eyes of 6 rats (6 eyes) were injected

intravitreally with 0.1 ml of a single dose of monosodium glutamate (40 nmol/ml) and at the same time, each rat received a single dose of taurine intraperitoneally as in taurine group (*Hong-xia et al., 2005*).

Intravitreal procedure

The pupil was dilated using Mydriacyl eye drops (Tropicamide 1%, Alcon pharmaceutical, USA) 10 minutes prior to the procedure. The technique of intravitreal injection in rats was performed according to (Chiu et al., 2007). The rats were generally anesthetized by intra-peritoneal injection of ketamine (50 mg/kg) and xylazine (8mg/kg) (volume ratio at 2:1) to minimize pain and discomfort. Additional anesthetic in the form of Benox eye drops (Benoxinate Hydrochloride 0.4%, Egyptian Int. Pharmacetical Industries CO., Egypt) was administered. The upper nasal part of the sclera was pierced 1.5 mm posterior to the limbus (pars plana) with a 30-gauge needle. The needle tip was visualized during the procedure to avoid retinal damage. Injection of the monosodium glutamate or BSS was performed into the posterior vitreous over a period of three minutes to avoid reflux. After intravitreal injection Tobrex eye drops (Tobramycin 0.3%, Alcon pharmaceutical, USA) was administered five times daily to avoid ocular infection. Three days after injection, all the animals were sacrificed and the eye balls were enucleated.

Histological processing

Samples from each group were fixed in 10% neutral-buffered formalin, dehydrated and processed for paraffin blocks. Sections (5 µm) were subsequently cut and stained with hematoxylin and eosin (H & E) (*Bancroft & Gamble, 2013*).

For semi-thin sections samples were immediately cut into cubes (1 mm in diameter) and fixed overnight in 2.5% phosphate-buffered glutaraldehyde (pH 7.3) at 4°C and processed for embed in fresh Epon capsules. Then sections 1 µm in thickness were cut with a glass knife and stained with toluidine blue (*Drury & Wallington, 1980*).

Immunohistochemical processing

Staining procedure using an immunoperoxidase technique was performed as previously described (*Bancroft & Gamble, 2013*). Serial retinal sections (3 µm) were deparaffinized, followed by endogenous peroxidase quenching with 3 % H_2O_2 / methanol for 5 minutes. Primary antibodies were applied overnight at 4° C followed by 1 hour incubation with a biotinylated secondary antibody at room temperature. Antibody binding was visualized by incubating the sections in diaminobenzidine. All incubations were performed in PBS. Finally, the sections were counterstained with hematoxylin, dehydrated, and covered.

Immunohistochemical staining for Anti-glial fibrillary acidic protein antibody was performed to the glial cells reactivity. This was conducted as described by (*Chen and Weber, 2002*). The sections were incubated with a primary rabbit polyclonal anti-GFAP antibody (1:1,000, 4°C, overnight; DAKO, Carpinteria, CA), then with a biotinylated goat anti-rabbit secondary antibody (DAKO).

Immunohistochemical staining for synaptophysin (SYN) was applied to detect the synaptic function. The steps were performed according to (*Dan et al., 2008*). The sections were incubated with a mouse SYN antibody (Chemicon, Temecula, CA), then with a biotinylated anti-mouse antibody (Vector Laboratories Inc., Burlingame, CA).

Immunohistochemical staining for vascular endothelial growth factor (VEGF) was performed to detect the vascular integrity and endothelial function. This was guided by the method of Youssef and Said (2014). Immunohistochemical staining for caspase-3 was performed according to (*Wu et al., 2002*) to demonstrate apoptosis. The sections were incubated with a primary rabbit antibody directed against cleaved caspase-3 (1:100; Cell Signaling Technology, Beverly, MA), then with a peroxidase-conjugated secondary antibody (1:200, Amersham, Buckinghamshire, UK).

All histological and immunohistochemical sections were examined and photographed using light microscope (Olympus 268 M microscope, Japan).

Histological morphometric analysis

Using the image analysis system Leica Q500 MC (Leica, Wetzlar, Germany) connected to an Olympus microscope (model BX51, Olympus Japan) equipped with digital camera for histological grading at (magnification X400), six non overlapping fields in six sections obtained from different animals from the same group were used for measuring. Total retinal thickness and the thickness of the outer nuclear layer (ONL) and inner nuclear layer (INL) were measured in µm in H&E stained sections (magnification X 400). The number of ganglion cells was counted in toluidine blue sections with a magnification X1000 and the average number was taken in each group.

Mean area % of each of GFAP, VEGF, SYN or caspase - 3 positive immunostaining: it was measured in immunostained sections (magnification X 400) using the color detect menu.

Statistical analysis

All data were collected and analyzed statistically using SPSS for windows version 13.0 (SPSS Inc., Chicago, USA). Quantitative data were expressed as mean and standard deviation. Qualitative data were expressed as number and percentage. One way analysis of variance (ANOVA) was employed to compare means between more than two groups with Bonferroni post Hoc test for comparison between groups if significant results were obtained. Independent sample -t test was used for comparison of quantitative variables between two groups. The significance of the data was determined by the probability (P - value). P-value $\leq$ 0.05 was considered statistically significant.

RESULT

Histological results

Examination of sections of the retina of control groups showed similar results. The sections showed normal arrangement of the different ten layers of retina, from outside inwards. Retinal pigment epithelium (RPE), photoreceptor layer (Ph), outer nuclear layer (ONL), outer plexiform layer (OPL), inner nuclear layer (INL), inner plexiform layer(INL), ganglion cell layer (GCL), and nerve fiber layer (NFL). RPE was formed of a single layer of cells with ill-defined cell boundaries, with pale, vesicular and oval in shape nuclei, and cytoplasmic pigment granules. Bruch's membrane and the underlying choroid capillaries were observed. The rods nuclei appeared round to oval, and deeply stained having dense chromatin filling most of the nucleus, while the cones nuclei appeared oval, fewer, and paler with peripheral chromatin aggregations. The inner nuclear layer (INL) with its four types of cells; horizontal cells, bipolar cells, Müller cells, and amacrine cells with its indented lightly stained nuclei was observed. The processes of the Müller cells were observed extending towards inner plexiform layer (IPL). Note the nerve fibers traversing the IP. Ganglion cell layer (GCL) was formed of a single row of large ganglion cells exhibiting round to oval, pale, and vesicular nuclei with prominent nucleoli. The cells were intermingled with nerve fibers (Fig.1). Total retinal thickness, ONL thickness & INL thickness (Tables 1- 3) respectivaly. Ganglion cell count (Table 4).

Also, retinal sections of taurine group showed almost a similar histological picture as the control group except that in most sections multiple blood vessels were present in the outer plexiform layer (OPL), the inner nuclear layer (INL), the inner plexiform layer (IPL), and the ganglion cell layer (GCL) (Fig. 2). Total retinal thickness, ONL thickness & INL thickness (Tables 1-3) respectivaly. Ganglion cell count (Table 4).

Examination of retinal sections of the glutamate group showed extensive damage and disruption of the histological structure of the retina (Fig. 3). There was a significant decrease in the total thickness of the retina. This was evidenced by morphometric results. The retinal pigment epithelium (RPE) was detached. The photoreceptor layer showed marked irregular appearance, vacuole formation, focal loss and wide retinal blood vessel was seen within the GCL. The outer nuclear layer (ONL) showed loss of discrimination between the rods and cones. The INL showed many cells with cytoplasmic vacuolation. Some cells have dark, pyknotic nuclei; some had nuclei with fragmented chromatin, while others had

dissolution of their nuclei and appeared as cell ghosts. Complete absence of ganglion cells and the presence of small dark glial cells within the GCL were noticed. Loss of clear discrimination between the four types of cells of INL, however increased number of Müller cells was observed with its processes traversed the IPL. Total retinal thickness, ONL thickness & INL thickness (Tables 1-3) respectivaly. Ganglion cell count (Table 4).

Examination of retinal sections of combined glutamate and taurine group showed a histological picture of the retina similar to that of the control group (Fig. 4). The total retinal thickness was preserved. The INL appeared with higher cell density and the four cell types of this layer could be differentiated with presence of cytoplasmic vaculation and blood vessel. Also, the IPL preserved its reticular appearance with no widening of the spaces between its fibers. The GCL was formed of a single row of ganglion cells with intact nerve fiber layer (NFL) and internal limiting membrane (ILM). Total retinal thickness, ONL thickness & INL thickness (Tables 1-3) respectivaly. Ganglion cell count (Table 4).

Immunohistochemical Results

• *Glial fibrillary acidic protein*

Examination of GFAP sections (Fig. 5) revealed the GFAP labeling was mostly localized in the astrocytes normally present in the GCL, the NFL and in some Müller glial cell end-feet in the control group and taurine group (Fig. 5 A, B). While, there was markedly increase extension of staining throughout the whole retinal thickness with marked intensity in the inner retinal layers observed in glutamate injected group (Fig. 5 C). Examination of combined glutamate and taurine group was closely resembled that of the control group (Fig. 5D).

A significant increase in the mean area % of GFAP was detected in glutamate group (21.76 7.86 SD) when compared to the control group (5.31 ± 1.45 SD) (*P*-value < 0.001). Mean area % of GFAP in taurine group was (5.08 ± 1.32 SD) and in the combined group was (6.15 ± 1.32 SD) with a significant decrease when compared with glutamate group (*P*-value < 0.001).

• *Synaptophysin*

As regards SYN immunostained sections (Fig. 6) a faint positive reaction in the OPL and the IPL in the control group and taurine group was noticed (Fig. 6 A, B), While there was marked increase in the IPL and the OPL which became heavily stained in glutamate group (Fig. 6 C). Staining of the IPL and OPL was less in combined glutamate and taurine than glutamate only injected group (Fig. 6 D).

A significant increase in the mean area % of SYN was detected in glutamate group (45.78 ± 29.15 SD) when compared to the control group (20.05 ±13.08 SD) (*P*-value < 0.001). Mean area % of GFAP in taurine group was (19.69 ±12.28 SD) and in the combined group was (22.56 ± 12.99 SD) with a significant decrease when compared with glutamate group (*P*-value < 0.001).

• *Vascular endothelial growth factor*

Examination of immunostained sections for VEGF revealed negative expression in all the retinal layers in the control group, taurine and combined glutamate and taurine groups (Fig. 7 A, B, D). While the retina showed faint positive VEGF immunoreaction distributed in most of retinal layers in glutamate group (Fig. 7C).

• *Caspase-3*

Examination of immunostained sections for caspase-3 showed no expression in all retinal layers including the ganglion cells in the control group, taurine and combined glutamate and taurine groups (Figs. 8 A, B, D). Also the immunoreaction appeared weak in glutamate group (Fig. 8 C).

Morphometric results:

• No statistically significant difference between control and Taurine groups regarding the mean total outer and inner retinal layers thickness (*P*-values = 1.0). On the other hand, there was a statistically significant decrease in glutamate group compared to the control groups (*P*-value < 0.001). Note that there was a

statistically significant increase in all parameters compared to glutamate group (P-value < 0.001).

• Statistically significant (P-value < 0.001) reduction in the mean number of ganglion cells in Glutamate group compared to the control groups.

• No statistically significant difference in the mean number of ganglion cells between contol and Taurine groups (P-value = 1.57). Also there was no statistically significant difference in the mean number of ganglion cells between combined group of Glutamate and Taurine compared to control groups (P-value = 1.05).

Table 1: Comparison between the total retinal thickness of different groups in µm.

Group	Mean	Standard deviation	*P* value
Control Group	111.12	± 0.07	
Glutamate Group	29.71	± 0.03	0.0001*
Taurine Group	111.11	± 0.07	1.001
Combined Glutamate & Taurine group	102.97	± 0.04	0.0001*

Table 2: Comparison between the outer nuclear layer thickness of different groups in µm.

Group	Mean	Standard deviation	*P* value
Control Group	24.21	± 0.03	
Glutamate Group	7.33	± 0.03	0.0001*
Taurine Group	24.21	± 0.03	1.001
Combined Glutamate & Taurine group	21.73	± 0.04	0.0001*

Table 3: Comparison between the inner nuclear layer thickness of different groups in µm.

Group	Mean	Standard deviation	*P* value
Control Group	14.99	± 0.03	
Glutamate Group	3.22	± 0.04	0.0001*
Taurine Group	14.98	± 0.07	1.001
Combined Glutamate & Taurine group	12.21	± 0.04	0.0001*

Table 4: Comparison between retinal ganglion cells of different groups.

Group	Mean	Standard deviation	*P* value
Control Group	12	± 0.89	
Glutamate Group	1.31	± 1.21	0.0001*
Taurine Group	11.87	± 1.26	1.57
Combined Glutamate & Taurine group	11.54	± 1.13	1.05

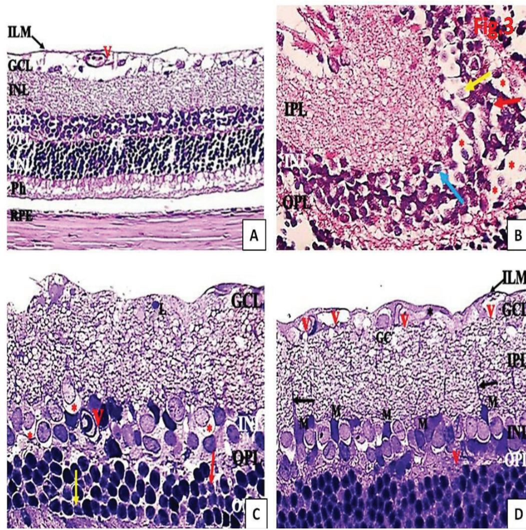

Fig. 1: A. A photomicrograph of a parasagittal section of a rat's retina (control group) showing normal arrangement of the different ten layers of retina, from outside inwards. RPE is formed of a single layer of cells with ill-defined cell boundaries, C. have pale and oval in shape nuclei (n). Note the Bruch's membrane (↑↑↑) and the underlying choroid capillaries (Ch). The rods nuclei (R) are round to oval, and deeply stained having dense chromatin filling most of the nucleus, while the cones nuclei (C) are oval, fewer, and paler with peripheral chromatin aggregations.D. The inner nuclear layer with its four types of cells; horizontal cells (H), bipolar cells (B), Müller cells (M), and amacrine cells (A) with its indented lightly stained nuclei. The processes of the Müller cells (arrow heads) are observed extending towards IPL. Note the nerve fibers traversing the IPL (↑↑↑). B. GCL is formed of a single row of large ganglion cells (GC) exhibiting round to oval, pale, and vesicular nuclei with prominent nucleoli. The cells are intermingled with nerve fibers (↑). NFL and ILM. A (H&E X400), B (Toluidine blue X400), C & D (Toluidine blue X1000).

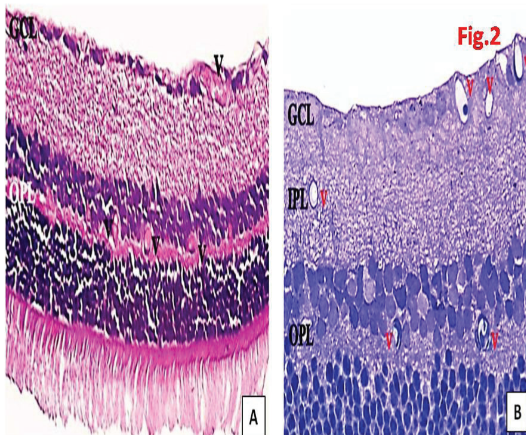

Fig. 2: A photomicrograph of a parasagittal section of a rat's retina (taurine group) showing; blood vessels (v) in OPL, IPL and GCL. A (H&E X400), B (Toluidine blue X1000).

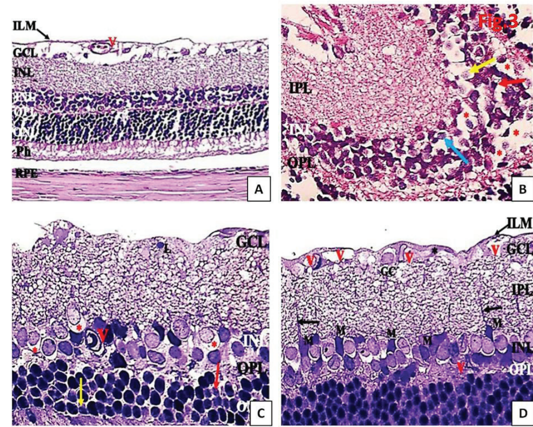

Fig. 3: A photomicrograph of a parasagittal section of a rat's retina (glutamate group) showing A; highly significant decrease in the total thickness of the retina, RPE is detached, The photoreceptor layer (Ph) shows marked irregular appearance, vacuole formation, and focal loss, wide retinal blood vessel (v) is seen within the GCL, B & C; ONL loss of discrimination between the rods and cones, INL shows many cells with cytoplasmic vacuolations (*). Some cells have dark, pyknotic nuclei (red arrow), some have nuclei which show fragmentation of their chromatin (blue arrow), while others show dissolution of their nuclei and appear as cell ghosts (yellow arrow). Note the complete absence of ganglion cells, and the presence of small dark glial cells (L) within the GCL,D; loss of clear discrimination between its four types of cells of INL, however, increased number of Müller cells (M) is observed with its processes (↑) traversed IPL. A (H&E X400), B (H&E X1000), C &D (Toluidine blue X 1000).

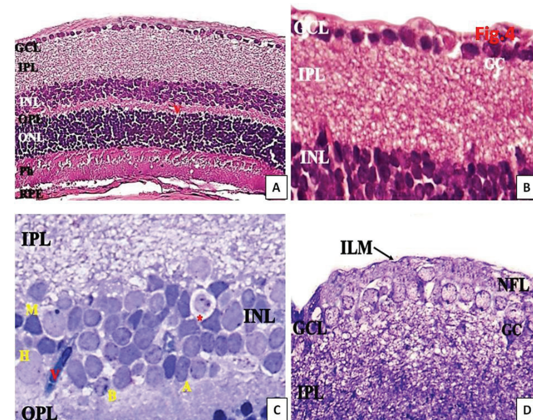

Fig. 4: A photomicrograph of a parasagittal section of a rat's retina (combined glutamate and taurine group) showing A; the total retinal thickness is apparently preserved, B & C; The INL appears higher cell density and four cell types of this layer could be differentiated with presence of cytoplasmic vacuolation (*) and blood vessel (v), IPL preserves its reticular appearance with no widening of the spaces between its fibers, D; GCL is formed of a single row of ganglion cells (GC), Note the preserved NFL, and the intact ILM. A (H&E X400), B (H&E X1000), C &D (Toluidine blue X1000).

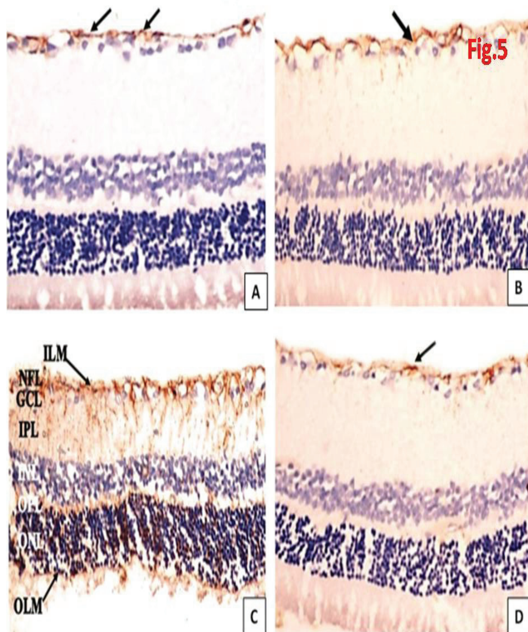

Fig. 5: A photomicrograph of a parasagittal section in a rat's retina showing GFAP immunohistochemical staining among the different groups; A; control group, B; taurine group, C; glutamate group, D; combined glutamate and taurine group (GFAP immunostaining X400).

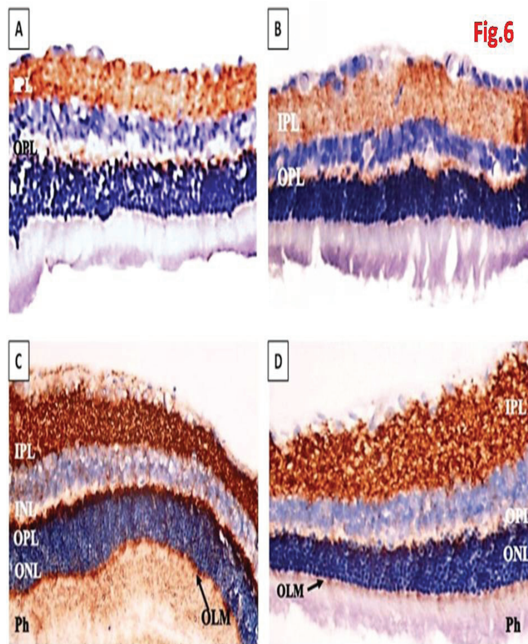

Fig. 6: A photomicrograph of a parasagittal section in a rat's retina showing synaptophysin (SYN) immunohistochemical staining among the different groups. A; control group, B; taurine group, C; glutamate group, D; combined glutamate and taurine group (SYN immunostaining X 400).

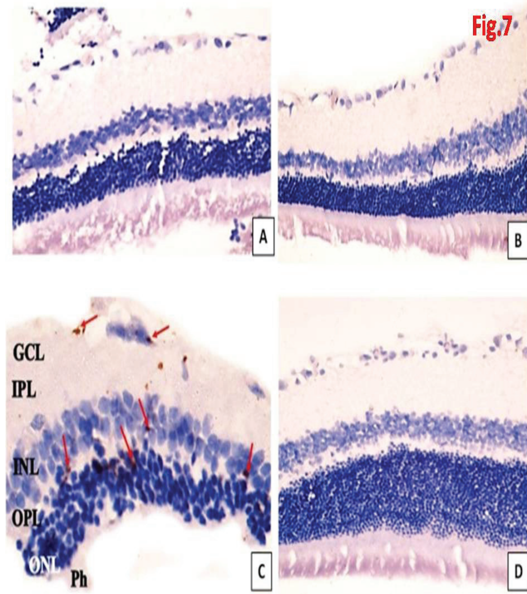

Fig. 7: A photomicrograph of a parasagittal section in a rat's retina showing VEGF immunohistochemical staining (red arrows refer to) among the different groups. A; control group, B; taurine group, C; glutamate group, D; combined glutamate and taurine group (VEGF immunostaining X400).

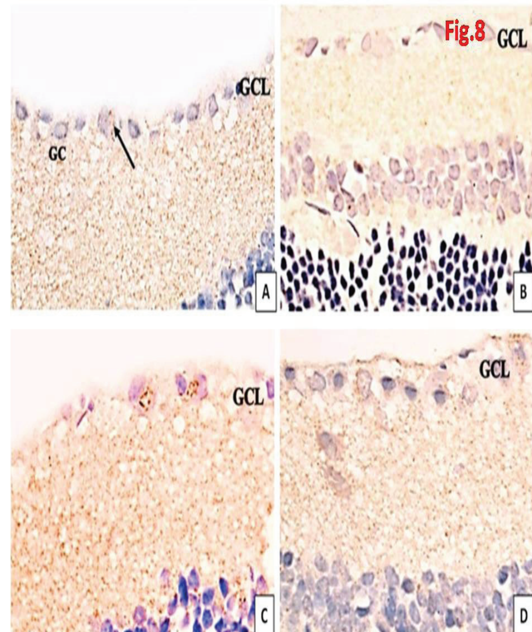

Fig. 8: A photomicrograph of a parasagittal section in a rat's retina showing caspase-3 immunohistochemical staining among the different groups A; control group, B; taurine group, C; glutamate group, D; combined glutamate and taurine group (Caspase -3 immunostaining X1000).

DISCUSSION

In case of increased glutamate levels (*Zeng et al., 2010*) or when glutamate clearance is insufficient, there will be acceleration of retinal cells death, which might play an important role in the pathogenesis of retinopathies (*Ishikawa, 2013*). (*Ripps and Shen 2012*) reported that glutamate - triggered neuronal damage is known to occur when the glutamate concentration of interstitial fluids reaches abnormally high levels as a result of hypoxia, ischemia, diabetes or brain trauma. They stated that a 30 minutes exposure to parenterally administered glutamate produced a histo¬pathological lesion characterized by swollen cell bodies in the GCL, the INL, and extending to the IPL. Even after washing and transferring the excised retina to glutamate-free medium, they found that the lesion had progressed further.

Since the outer blood retinal barrier is formed by the tight junctions between the retinal epithelial cells (*Cunha-vas, 2004*) therefore damage to this layer as recorded in this study might lead to excess glutamate diffusion in the extracellular spaces between the retinal cells. This is extremely harmful to the retinal metabolic equilibrium and accelerates the degenerative process in the retina leading to compromised retinal function and eventual blindness.

(*Lu et al. 2003*) reported that the photoreceptor nuclei decreased from eight rows to three or six rows in the retina of spontaneously diabetic fatty rats. Moreover, (*Szabadfi et al. 2012*) noticed degeneration of cones with decrease in the number of the photoreceptors nuclei in the retina of diabetic rats as a result of elevated glutamate level. Also they noticed degenerative changes in cells of the INL in the glutamate diabetic retinopathy. Moreover, (*Nag et al. 2011*) suggested that these changes occurred due to the effect of oxidative stress.

The extensive reduction and affection of the ganglion cells may be attributed to the presence of apoptosis in the ganglion cells caused by the glutamate toxicity. As regard the NFL, it showed thinning out, up to either partial or total loss. This can be explained as a predictable finding secondary to the RGC death. Glutamate specifically targeted the ganglion cells which are the final output neurons of the retina that collect the electrical messages concerning the visual signals from all the preceding layers to the optic nerve. This was in agreement with (*Yamagishi and Aihara 2014*) who found that under glutamate stress, ganglion cells viability was reduced to 58%.

In the present study, the histologic variations affecting retinal layers were verified by morphometric measurements. There was a statistically significant decrease in the mean total, outer, inner retinal thickness and ganglion cell counts of glutamate group as compared to the control, which could be attributed to the degenerative changes that involved the sensory retina.

Neovascularization (NVs) of the retina was shown in the glutamate group in the OPL, the INL while in the GCL, the blood vessels became more encountered than those found in the control group. NVs might result from retinal ischemia in response to glutamate toxicity in an attempt to supply blood to the affected areas, the retina responds by growing new blood vessels. This was in agreement with (*Hassan et al. 2012*) who noticed growth of new vessels in the diabetic retina and referred the occurrence of NVs to the extensive lack of oxygen in the retinal capillaries of diabetics.

Examination of retinal sections of the taurine treated group showed almost same results and same integrity of the retinal layers and cells as the control group, except that in most sections there was marked blood vessels appearance. The vessels appeared in the OPL, IPL, extending to the INL and the GCL.

Coadministration of taurine with glutamate showed a histological picture of the retina more or less similar to that of the control group. These findings suggest that taurine initiates a new protective approach against glutamate-induced toxicity, and is mandatory to protect against retinal damage.

Concerning the mechanism by which taurine produces its protective effect on the retinal tissue, (*Warskulat et al. 2007*) reported that taurine is one of the compatible organic osmolytes that may protect cells from apoptosis

in various in vitro and in vivo models. (*Duan et al. 2007*) documented that taurine inhibited the glutamate-induced increase of [Ca +2], and added that this inhibition is immediate and long-lasting. In addition, Leon et al. (2009) suggested that the mode of action of taurine to maintain Ca +2 homeostasis under neuronal excitation is due to its action of preventing glutamate-induced membrane depolarization. (*Chen et al. 2009*) reported that the cytoprotective role of taurine has been extended to preserving the integrity of mitochondrial pore permeability and thus they proved that taurine protects against hypoxia-induced apoptosis by preventing mitochondrial dysfunction. Moreover, (*Marcinkiewicz and Kontny 2014*) reported that the beneficial effects of taurine are as a result of its antioxidant properties.

Nevertheless, few structural changes were demonstrated in this group which received taurine with glutamate. Blood vessels appeared in the OPL layer and some extended into the INL. These blood vessels were most probably formed under the effect of taurine, increasing the blood perfusion to the retinal tissue. This interesting finding was supported by (*Baek et al. 2012*) who proved that taurine had the ability to promote angiogenesis by increasing proliferation, migration, and tube formation of endothelial cells by activating angiogenesis-associated signal pathways without increasing VEGF expression and without affecting vascular inflammation or vessels permeability as the cell integrity of the surrounding cells are preserved to a great extent and no signs of tissue edema were detected. The expressional amount, molecular size, and phosphorylation status of GFAP are known to be changed in a variety of pathological conditions (*Takemura et al. 2002*).

In the present study, all immunohistochemical stained sections of the retina of the rats of control group and of taurine group were similar. The GFAP labeling was mostly localized in the astrocytes normally present in the GCL, NFL and in some Müller glial cell end-feet that are incorporated into the ILM. Other layers showed negative GFAP immunohistochemical staining. Similarly, (*Gaucher et al. 2012*) mentioned that in control animals, GFAP expression was limited to the ILM. Also, (*Zhou et al. 2014*) reported that in healthy adult rat retinae, GFAP was primarily confined to the somas of astrocytes in NFL and GCL.

While in glutamate group, GFAP immunohistochemical staining was markedly increased as heavily stained processes could be seen extending throughout the whole retinal thickness with marked intensity in the inner retinal layers. These findings were supported by (*Lewis and Fisher 2003*) who declared that since normal human Müller cells radial fibers are GFAP negative, therefore, the specificity of the GFAP marker ensures that, when staining occurs, it is a reliable indicator of the presence of reactive glial cells in the tissue. Moreover, (*Sethi et al. 2005*) and (*Xue et al. 2006*) reported that in pathological retinal conditions such as glaucoma, ischemia, chronic hypoxia, diabetic retinopathy, light damage and retinal detachment, GFAP is vigorously expressed in Müller glial cells.

Demonstration of GFAP down regulation with taurine coadministration with glutamate revealed that application of taurine prevented the expression of detectable levels of GFAP by Müller cells. GFAP expression was restricted to GCL, NFL and ILM. This implies a neuroprotective role of taurine in the retina by preventing reactive retinal gliosis. This finding was supported by (*Zeng et al. 2010*) who found that treatment with taurine caused an elevation of taurine content and a decline in levels of glutamate in retina and blocked the increased expression of GFAP. Also, (*Ito et al. 2012*) reported that taurine supplementation after diabetic onset effectively improved the changes in ultrastructure and attenuated induction of GFAP in the retina of diabetic rats indicating the beneficial role of taurine on diabetic retinopathy.

In rat retina, synapses in the OPL and IPL play an important role in visual signal transmission. Changes of synaptic number or structure in these two layers will affect the visual function. Retinal injury may cause synapse alteration which occurs earlier than changes in cell body of neurons (*Dijk et al. 2007*). SYN is an integral membrane protein of the synaptic vesicles and it is widely used as one of the synaptic function markers (*Dan et al. 2008*).

In this present study, retinal examination of rats of control and taurine groups showed

the same results, where immunohistochemical staining for SYN revealed a faint positive reaction in the OPL and the IPL. This finding was supported by (*Dan et al. 2008*). In glutamate group, there was an apparent marked increase in SYN expression in the IPL and the OPL. It also extended between the cells of both the INL and the ONL, reaching the OLM (outer limiting membrane) and even to the photoreceptor layer. (*McKay et al. 2009*) found that following retinal injuries in rats, the SYN expression in the retina was detected in the IPL, OPL and the OLM with strong staining of nerve processes traversing the other layers of the retina. The co-administration of taurine with glutamate has shown reduction in the expression of SYN when compared to the glutamate group which supports the protective effect of taurine on the diseased retina.

Detection of VEGF was done as it was considered not only as a proangiogenic marker but also a detector of the occurrence of vascular permeability and breakdown of the blood retinal barrier as mentioned by (*Kusari et al. 2007*). The retina showed negative VEGF immunostaining in all its layers in control and taurine treated groups. (*Wurm et al. 2008*) documented that the expression of VEGF is increased under ischemic-hypoxic, oxidative stress and pathological conditions. They stated that VEGF exerts both neuroprotective and detrimental effects. It inhibits the swelling of glial cells. The VEGF-evoked release of glutamate may contribute to the excitotoxic swelling of retinal neurons after ischemia. In addition, (*Deissler et al. 2013*) proved a dominant role of VEGF in the disturbance of the blood-retinal barrier in diabetic rat's retina. (*Ito et al. 2012*) suggested that taurine may normalize the retinal vascular function in diabetes as taurine supplementation attenuated the induction of retinal VEGF, which was associated with vascularization in diabetic rat retina. (*Zeng et al. 2009*) reported that normal control retina showed low level of VEGF expression. The observation in the present study that taurine had angiogenic activity was proved by Baek and his colleagues (2012), they found that its angiogenic activity is associated with an increase in endothelial cell proliferation by promoting cell cycle progression as well as elevation of cell migration. They proved that taurine promoted angiogenesis without increasing VEGF expression. This amino acid has been also shown to increase endothelial function via the up-regulation of endothelial nitric oxide synthase and nitric oxide production, which are important for vascular remodeling. These observations indicate that taurine can exert pharmacological means to control the functions of the vasculature and endothelial cells (*Fennessy et al. 2003*).

Caspases are important mediators of neuronal apoptosis. They play a pivotal role in developmental and pathologic death in the nervous system. Caspase activation has been demonstrated in the retina in a number of degenerative model of animals in which apoptosis was caused by ischemia, axotomy, excitotoxicity, and gene mutations. Caspase-3 is considered one of the most commonly activated caspases in apoptosis (*Wu et al. 2002*).

(*Kermer et al. 1999*) stated that at advanced stage of cell death, single cells shortly show activation of caspase-3 followed by a time-window in which caspase-3 labeling diminishes and the cells dies apoptotically. Moreover, (*Wu et al. 2002*) reported that the wave of apoptosis reached its maximum at 8 to 16 hours and declined at 24 hours after exposure to light. (*Taranukhin et al. 2010*) proved that taurine is a strong modulator of apoptosis and is widely known to prevent elevated levels of caspases, calpains, and pro-apoptotic proteins such as Bad, Bax, and Bim. They added that taurine significantly reduce apoptotic death by down regulating the activities of caspase-3 and intracellular calcium.

In conclusion taurine is protective to the retina against glutamate-induced toxicity. This endogenous amino acid could to a great extent preventing most of the deleterious effects caused by glutamate on the structure of the retina. The present results could have clinical implications in protecting the RGCs and reducing glutamate-induced toxicity in several ophthalmic diseases as glaucoma, diabetic retinopathy and retinal ischemia as in central retinal artery occlusion.

ACKNOWLEDGEMENT

We would like to thank Dr. Ahmed Mohamed Abdellah Vet. Surgeon, Ass. Lecturer of Clinical Pathology at Medical Research Centre, Faculty of Medicine, Ain shams University for his support and great help in conducting this animal research.

REFERNECES

Babai, N., Atlasz, T., Tamás, A. ,et al., .2005. Degree of damage compensation by various PACAP treatments in monosodium glutamate-induced retinal degeneration. Neurotoxicity Research, 8(3-4):227–233.

Baek, Y.Y., Cho, D.H., Choe, J. et al. 2012. Extracellular taurine induces angiogenesis by activating ERK-, Akt-, and FAK-dependent signal pathways. European Journal of Pharmacology, 674 (2-3):188 -1 99.

Bancroft, J. and Gamble, M. 2013. Bancroft's theory and practice of histological techniques, 7th edition, Edinburgh. Churchill Livingstone Pub 2013.

Buddhala, C., Prentice, H., Wu, J. 2012. Modes of action of taurine and granulocyte colony-stimulating factor in neuroprotection. Journal of Experimental and Clinical Medicine, 4 (1): 1-7.

Chen, H. and Weber, A. 2002. Expression of glial fibrillary acidic protein and glutamine synthetase by Müller cells after optic nerve damage and intravitreal application of brain-derived neurotrophic factor. Glia, 38 (2):115 - 125.

Chen, K., Zhang, Q., Wang, J. et al. 2009. Taurine protects transformed rat retinal ganglion cells from hypoxia-induced apoptosis by preventing mitochondrial dysfunction. Brain Research. 1279: 131 - 138.

Chiu, K., Chang, R.C. and So, K.F. 2007. Intravitreous injection for establishing ocular diseases model. Journal of Visualized Experiments, (8):313.

Cunha-Vaz, J.G. 2004. The blood-retinal barriers system. Basic concepts and clinical evaluation. Experimental Eye Research, 78(3):715 - 721.

Dan, C., Jian-Bin, T., Hui, W., et al. 2008. Synaptophysin expression in rat retina following acute high intraocular pressure. Acta Histochemica Cytochemica,41(6): 173 - 178.

De´nes, V., Lakk, M., Czotter, N. and Gabriel, R. 2011. A precise temporal dissection of monosodium glutamate- induced apoptotic events in newborn rat retina in vivo. Neurochemical Research, 36 (8): 1464 - 1474.

Deissler, H., Deissler, H., Gerhard, K., et al. 2013. VEGF but not PlGF disturbs the barrier of retinal endothelial cells. Experimental Eye Research, 115: 162 - 171.

Dijk, F., Bergen, A.A., and Kamphuis, W. 2007. GAP-43 expression is upregulated in retinal ganglion cells after ischemia reperfusion-induced damage. Experimental Eye Research, 84 (5): 858 - 867.

Drury, R. and Wallington, E. 1980. Carleton's Histological techniques, 5th edition, Oxford University Press, New York , 237.

Duan, Y., Gross, R.A. and Sheu S.S. 2007. Ca+2 dependent generation of mitochondrial reactive oxygen species serves as a signal for poly (ADP-ribose) polymerase-1 activation during glutamate excitotoxicity. Journal of Physiology, 585: 741 - 758.

Fennessy, F.M., Moneley, D.S., Wang, J.H., et al. 2003. Taurine and vitamin C modify monocyte and endothelial dysfunction in young smokers. Circulation , 107 (3): 410–415.

Gaucher, D., Arnault, E., Husson, Z. et al. 2012. Taurine deficiency damages retinal neurons: cone photoreceptors and retinal ganglion cells. Amino Acids, 43 (5): 1979 - 1993.

Hassan, S.S., Bong, D.B.and Premsenthil, M. 2012. Detection of neovascularization in diabetic retinopathy. Journal of Digital Imaging, 25 (3): 437 - 444.

Hernández, C, and Simó, R. 2012. Neuroprotection in diabetic retinopathy. Current Diabetes Reports, 12 (4): 329 - 337.

Hong-xia X., Man-tian M., Jun-dong Z., et al., 2005. Effects of glutamate excitotoxicity and taurine intervention on retinal expression of glial fibrillary acid protein in rats. Acta Academiae Medicinae Militaris Tertiae, 27(11): 1078-1082.

Ishikawa, M. 2013. Abnormalities in glutamate metabolism and excitotoxicity in the retinal diseases. Scientifica : 1-13.

Ito T, Schaffer S and Azuma J. 2012. The potential usefulness of taurine on diabetes mellitus and its complications. Amino Acids, 42 (5): 1529 - 1539.

Kermer, P., Nikolaj, K., Monika, L., et al. 1999. Activation of caspase-3 in axotomized rat retinal ganglion cells in vivo. FEBS Lett. 453 (3): 361 - 364.

Kusari, J., Zhou, S., Padillo, E., et al. 2007. Effect of memantine on neuroretinal function and retinal vascular changes of streptozotocin - induced diabetic rats. Investigative Ophthalmology and Visual Science, 48 (11): 5152 - 5159.

Leon, R., Wu, H., Jin, Y. et al. 2009. Protective function of taurine in glutamate induced apoptosis in cultured neurons. Journal of Neuroscience Research, 87: 1185 - 1194.

Lewis, G.P.and Fisher, S.K. 2003. Upregulation of glial fibrillary acidic protein in response to retinal injury: its potential role in glial remodeling and a comparison to vimentin expression. International Review of Cytology, 230: 263 - 290.

Lu, Y., Bhutto, A. and Amemiya, T. 2003. Retinal changes in Otsuka Long-Evans Tokushima fatty rats (spontaneously diabetic rat) - possibility of a new experimental model for diabetic retinopathy. Japanese Journal Ophthalmology , 47(1): 28 - 35.

Marcinkiewicz, J. and Kontny, E. 2014. Taurine and inflammatory diseases. Amino Acids, 46 (1): 7-20.

McKay, J.S., Steele, S.J., Ahmed, G., et al. 2009. An antibody panel for immunohistochemical analysis of the retina in Davidson's - fixed, paraffin - embedded eyes of rats. Experimental and Toxicologic Pathology , 61 (2): 91-100.

Nag, T., Wadhwa, S., Alladi, P., et al. 2011. Localization of 4-hydroxy 2 nonenal immunoreactivity in aging human retinal Müller cells. Annals of Anatomy, 193 (3): 205 - 210.

Ripps, H. and Shen, W. 2012. Taurine: a “very essential” amino acid. Molecular Vision, 18: 2673 - 2686.

Sethi, C.S., Lewis, G.P., Fisher, S.K., et al. 2005. Glial remodeling and neural plasticity in human retinal detachment with proliferative vitreoretinopathy. Investigative Ophthalmology and Visual Science, 46 (1): 329 - 342.

Standring S. Gray’s anatomy. 2008. The anatomical basis of clinical practice, 41th edition, Elsevier, London; 688 - 697.

Szabadfi, K., Atlasz, T., Kiss, P., et al. 2012. Protective effects of the neuropeptide PACAP in diabetic retinopathy. Cell and Tissue Research, 348 (1): 37-46.

Takemura, M., Nishiyama, H. and Itohara, S. 2002. Distribution of phosphorylated glial fibrillary acidic protein in the mouse central nervous system. Genes Cells, 7(3): 295 - 307.

Taranukhin, A., Taranukhina, E., Saransaari, P., et al. 2010. Neuroprotection by taurine in ethanol-induced apoptosis in the developing cerebellum. Journal of Biomedical Science, 24: 17 suppl 1:S12.

Warskulat, U., Borsch, E., Reinehr, R., et al. 2007. Taurine deficiency and apoptosis: findings from the taurine transporter knockout mouse. Archive of Biochemistry and Biophysics, 462 (2): 202 - 209.

Wu J, Gorman A, Zhou X, et al. 2002. Involvement of caspase-3 in photoreceptor cell apoptosis induced by in vivo blue light exposure. Investigative Ophthalmology and Visual Science, 43 (10): 3349 - 3354.

Wu, J. and Prentice, H. 2010. Role of taurine in the central nervous system. Journal of Biomedical Science, 17(1): 1 - 6.

Wurm, A., Pannicke, T., Wiedemann, P., et al. 2008. Glial cell-derived glutamate mediates

autocrine cell volume regulation in the retina: activation by VEGF. Journal of Neurochemistry ,104 (2): 386 - 399.

Xue, L., Lu, J., Cao, Q., et al. 2006. Müller glial cells express nestin coupled with glial fibrillary acidic protein in experimentally induced glaucoma in the rat retina. Neuroscience, 139 (2): 723 - 732.

Yamagishi, R. and Aihara, M.2014. Neuroprotective effect of astaxanthin against rat retinal ganglion cell death under various stresses that induce apoptosis and necrosis. Molecular Vision, 20: 1796 - 1805.

Youssef, N. and Said, A. 2014. Immunohistochemical expression of CD117 and vascular endothelial growth factor in retinoblastoma: possible targets of new therapies. International Journal of Clinical and Experimental Pathology , 7(9): 5725 - 5737.

Zeng K., Xu H., Chen K.,et al. 2010. Effects of taurine on glutamate uptake and degradation in Müller cells under diabetic conditions via antioxidant mechanism. Mollecular and Cellular Neuroscience, 45 (2): 192- 199.

Zeng, K., Xu, H., Mi, M. et al. 2009. Dietary taurine supplementation prevents glial alterations in retina of diabetic rats. Neurochemical Research 2009; 34 (2): 244 - 254.

Zhou L, Wang H, Luo J, et al. 2014. Regulatory effects of inhibiting the activation of glial cells on retinal synaptic plasticity. Neural Regeneration Research, 9 (4): 385 - 393.

الدور الوقائى العصبي المحتمل للتورين علي خلايا العقد العصبيه بشبكية عين الجرذ من السميه المحدثه بمادة الجلوتاميت

كريمان محمد عبد الفتاح الجوهري*، إبتسام أحمد بهي الدين*، إيمان كمال محمد حبيب*، شيرين عادل سعد*، هاجر يسري راضي*، عزة محمد أحمد سيد**

*قسم التشريح والأجنة – كلية الطب – جامعة عين شمس

** قسم جراحة طب العيون – كلية الطب – جامعة عين شمس

ملخص البحث

الهدف من الدراسة: مادة الجلوتاميت من المواد التى تؤدى الى تحلل خلايا العقد العصبية بشبكية العين. وكان الهدف من العمل الحالى هو استقصاء الدور الوقائى العصبى المحتمل لمادة التورين على خلايا العقد العصبية بشبكية عين الجرذ الأبيض ضد السمية المحدثة بمادة الجلوتاميت.

المواد و الطرق: استخدم فى هذه التجربة عدد أربعة وعشرون من ذكور الجرذان (سبراج داولى) البيضاء البالغة والتى تم تقسيمهم الى أربع مجموعات. المجموعة الأولى وهى المجموعة الضابطة :تم حقن العين اليمنى للجرذان داخل الجسم الزجاجى بجرعة ٠,١ ملليلتر من محلول متوازن الملح المجموعة الثانية وهى المجموعة المعالجة بمادة الجلوتاميت أحادى الصوديوم :تم حقن جرعة واحدة من مادة الجلوتاميت (٤٠ نانومول) مذابة فى محلول الملح داخل الجسم الزجاجى. المجموعة الثالثة وهى مجموعة التورين والتى تم حقنها بداخل الصفاق بجرعة واحدة من مادة التورين (٢٥ مجم لكل كجم من وزن الجسم) بعد اذابتها فى محلول الملح. المجموعة الرابعة وهى المجموعة المعالجة بكل من مادة الجلوتاميت ومادة التورين: تم حقن التورين (25 ملغم / كغم) مرة واحدة داخل الصفاق وفي الوقت نفسه تم حقن 0.1 مل من الجلوتاميت داخل الجسم الزجاجى. تم التضحية بالجرذان فى اليوم الثالث بعد عملية الحقن والحصول على مقلة العين وتشريح شبكية العين وتثبيتها وتمريرها للحصول على قوالب البارافين و مقاطع شبه رقيقه و تم عمل صبغة الهيماتوكسلين والايوسين و صبغات هستوكيميائية مناعية لإظهار البروتين الحمضى الليفى الدبقى والسينابتوفيزين ومعامل نمو الغشاء المبطن للأوعية الدموية أو الكسباس ٣ تم فحص جميع القطاعات وتصويرها عن طريق الميكروسكوب الضوئى.

النتائج: تم فحص شبكية العين فى المجموعات ووجد أن شبكية عين الجرذان فى المجموعه الأولى ا لضابطة والثالثه أعطت نفس النتائج 0 بينما تم العثور على أضرار واسعة النطاق واختلال بنية شبكية العين مع انخفاض ملحوظ في قياس السمك الكلى لشبكية العين نقصا ذا دلالة إحصائية عالية فى شبكية عين الجرذان فى المجموعة الثانية بمقارنتها بمثيلتها فى المجموعة الضابط و أظهرت طبقة الأنوية الداخلية و طبقة الأنوية الخارجية انخفاضا فى كثافة الخلايا بينما لوحظ تحسن ملحوظ فى اجمالي عدد الخلايا في شبكية العين بعد الحقن المادتيم معا (التورين و الجلوتاميت) بالإضافة إلى الحفاظ على هوية البناء المعمارى لطبقات الشبكيه المتعدده بينما ظهر سمك شبكية العين للمجموعة الرابعة مماثل للمجموعة الضابط ولكن ظهرت بعض الأوعية الدموية فى معظم القطاعات فى الطبقة الضفيرية الخارجية و طبقة الأنوية الداخلية و الطبقة الضفيرية الداخلية و طبقة خلايا العقد العصبية فى المجموعة الثالثة. وقد شهدت زيادة كبيرة في الصبغات الهستوكيميائية المناعية المختلفة بشكل واضح فى المجموعة الثانية المعالجة بمادة الجلوتاميت بمقارنتها بمثيلاتها فى المجموعة الضابطة. أما فى المجموعة الرابعة فقد ظهرت هذه الصبغات المختلفة مشابهة لنمط الصبغات فى المجموعة الضابطة أو أظهرت إنخفاضا فى حدة الصبغة مع عدم أو ضعف تلطيخ في المجموعات الأخرى

الخلاصة: أن مادة التورين تعد بمثابة مدخل جديد للوقاية العصبية لخلايا العقد العصبية بشبكية العين ضد السمية المحدثة بمادة الجلوتاميت و التى قد تؤدي بكثره في العديد من أمراض العيون مثل الجلوكوما واعتلال الشبكية السكري.