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ORIGINAL ARTICLE

Effect of Antiepileptics on Retinal Blood Flow in Adults Wister Rats

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ABSTRACT

Background: Carbamazepine (CBZ) is an effective antiepileptic drug (AED) used as first line of drug for of partial and convulsive generalized epilepsy in all age groups. Most common clinically reported side effect of CBZ is blurry or double vision. How it affects retinal histological architecture has not been explored yet.
Aim: To observe the light microscopic retinal changes in adult Wistar albino rats after administration of CBZ.
Methods: The experiment was conducted in University of Health Sciences, Lahore after the approval of ethical review committee. 18 Rats were randomly divided into 3 groups of 6 rats each. Group A served as control and was given 1ml saline alkalinized with 0.1N NaOH, Group B&C were given CBZ 50 mg/kg and 100 mg/kg respectively dissolved in 1ml saline alkalinized with 0.1N NaOH in 3 divided doses intraperitoneally for 7 days. Animals were sacrificed on 8th day, and retinal tissue sections of 5µm were stained for H& E to look for vascular congestion in retinal layers (yes/no). Chi2 was applied for statistical analysis using SPSS v 20.
Practical implications: ophthalmic examination mandatory for epileptic patients on long term use of AED.
Results: vascular congestion was seen among layers of retina in dose dependent manner in experimental groups (p=0.006)
Conclusion: The findings of the present study indicates that CBZ affects retina blood flow, can impose a potential risk to visual disturbances.
Key words: vascular congestion, epilepsy, carbamazepine (CBZ)

INTRODUCTION

Epilepsy (EP) is a neurological disorder, which has become a major public health issue requiring a comprehensive response. This disease affects people of all ages, genders, ethnicities, and social backgrounds, irrespective of geographic locations. The disease burden of epilepsy in Pakistan is estimated to be 9.99 per 1000 of the population. Disease prevalence is highest among people under 30 years of age(Khatri et al., 2003)

International League against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE) collectively define epilepsy and epileptic seizures, as "An epileptic seizure is a transient occurrence of signs and/or symptoms due to abnormal excessive or synchronous neuronal activity in the brain." The definition of epilepsy is definite when there is episode of at the least one episode of epileptic seizure **(Fisher et al., 2005)**.

Antiepileptic drugs (AED) are chronically administered with the intent of to put an end to the occurrence of epileptic seizures in a person at risk (Goldenberg, 2010). More or less 75% patients have been seen to successfully withdrawn from epileptic therapy after being seizure free for two to five years and therefore they can be removed from pharmacotherapy (Goldenberg, 2010).. The therapy for epilepsy is long-term. It requires consistent treatment with single (monotherapy) or combination of drugs (add-on or polytherapy). The disadvantages and advantages of prescribed AED should be kept in mind.

CBZ is commonly used antiepileptic drug, it is tricyclic compound chemically related to tricyclic antidepressants (TCAs). Walter Schindler in Switzerland discovered carbamazepine (C16 H12 N2 O) in 1953. (Tolou-Ghamari et al., 2013).This drug was initially known to treat trigeminal neuralgia but later in 1974 it was approved as an anticonvulsant and antiepileptic in the US(Grzesiak et al., 2003). It is highly valuable against partial seizures but known to worsen juvenile myoclonic epilepsy and various types of generalized seizure disorders, such as absence seizure (Tolou-Ghamari et al., 2013, Valamanesh et al., 2013, Kramer and Cash, 2012).

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Chronic antiepileptic drug treatment both with established and new agents is almost always associated with apparent alterations of visual function (Steinhoff et al., 1997) (Dereci et al., 2015). Visual defects like blurred vision, diplopia, diminished contrast sensitivity, dark adaptation, visual acuity and ophthalmoplegia, deficits in color-perception and loss of visual field are few of specific visual predicaments associated with AEDs therapy (Bayer et al., 1995, Nousiainen et al., 2000).

The survival and operation of retinal cells is directly related with ocular blood flow. Consequently, any decrease in blood flow to retina will have direct implications on visual function. Number of AEDs are known to decrease cerebral blood flow and/or cerebral metabolic rate for glucose (Theodore, 1988, Spanaki et al., 1999).Research studies inquiring about the consequences of systemic drugs therapy or vasoactive stimuli on cerebral and ocular blood flow in normal subjects have reported equivalent alteration in blood flow of brain and eye (Harris et al., 1994, Kiss et al., 1999). Thus, it is probable that a reduction in cerebral blood flow, formerly reported in patients on AED therapy, is imitated in the eye circulation. Therefore, both the epilepsy disease and the AED treatment affect circulation and the prospective harm can be exacerbating by ischemia. In few experimental hypoxic-ischemic conditions in addition to elevated intraocular pressure(Garcia-Valenzuela et al., 1995, Quigley et al., 1995) and glaucoma (Quigley, 1999, Qu et al., 2010, Kaur et al., 2008) necrotic (Buchi, 1992, Joo et al., 1999) and apoptotic changes in RGCs have been suggestive of that ischemia is directly or indirectly involved in retinal damage.

MATERIAL AND METHODS

The study is Experimental Randomized Controlled Trial study. The study was conducted in research laboratory of University of Health Sciences, Lahore. Eighteen-albino adult Wistar rats, of either gender, 180-200g weight were procured from animal house of University of Health Sciences. Before starting the experiment all animals were weighed and inspected thoroughly. The animals were divided in 3 groups, each of group having 6 animals. The

animals were housed in separate cages through entire length of experiment. The experimental animals were kept in controlled temperature of 20±0.5 °C, humidity 50±0.5, 12 hours dark and light cycle and fed on standardized diet. All experimental protocols were conducted in accordance with rules and regulations of ethical committee of University of Health Sciences, Lahore. After one week of acclimatization, experiment was started.

Groups of Animals: Rats were randomly divided into 3 groups, each having 6 rats. The-sampling technique was random sampling using balloting method.

Doses and frequency of carbamazepine given to the animals

Table1:

Group	Intervention	Frequency and Route of Administration	Duration	Day of Dissection
Group A n=6	1ml of saline alkalinized with 0.1 NaOH thrice daily	Three divided doses daily after 8 hours Intraperitoneal	7 days	24 hours after last dose
Group B n=6	50 mg/kg body weight thrice daily in 3 divided does CBZ (16.6 mg/kg) dissolved in 1ml of saline alkalinized with 0.1 NaOH	Three divided doses daily after 8 hours Intraperitoneal	7 days	24 hours after last dose
Group C n=6	100mg/kg per body weight thrice daily in 3 divided does CBZ (33.3mg/kg) in 1ml of saline alkalinized with 0.1 NaOH	Three divided doses daily after 8 hours Intraperitoneal	7 days	24 hours after last dose

Steps of Intraperitoneal Administration: Animal in cages were vigilantly withdrawn and controlled in head-down position appropriately. Anatomical landmarks were identified to accurately inject abdomen area; the lower right quadrant of the abdomen is recommended injection site for IP administration. The injection needle was inserted into the right lower quadrant of the abdomen at an angle of 30-40 to the horizontal directed towards the head to prevent the damage to the under lying organs. The entire length of injection needle was inserted within abdominal cavity (Shimizu, 2004).

Dissection of Animals: At the 8th day of experiment, animals were euthanized one by one with chloroform. The animals were decapitated. Orientation sutures were placed at the points of the external and internal canthi, on the conjunctiva. Eyes were enucleated by pressing against the canthi and pulling eyeballs out with forceps. For the fixation material to have greatest infiltration in tissues, a 0.1- to 0.2-mm deep incision was made approximately 1 mm posterior to the point of junction of retina and ciliary body. A small portion of optic nerve was kept intact with each eyeball

Tissue Preparation and Staining: After dissection, 10% formalin was used to fix the eyecups acquired from the rats for 48 hours. Next was fixation with Bouine solution for subsequent 48 hours. Tissues were washed out with 70% alcohol to clear Bouine solution (Nassar et al., 2015).Tissue pieces were discretely placed in individual marked tissue cassettes. The tissues was processed (Sakura TEC- 5EMJ). Ethanol in ascending grades of 70%, 90%, 95% and 100% concentration were used for tissue dehydration.

Embedding: Subsequently, the tissue pieces were cleared in xylene and infiltrated with molten paraffin wax of melting points 56-58ºC. Embedding station was used for making paraffin blocks.

Microtomy: Once the blocks were solidified, they were removed from the trough and extra paraffin was trimmed from edges. Before starting microtomy, the tissue blocks were positioned in freezer for 30 mins. Tissue orientation in paraffin blocks was coronal and sectioning started from optic nerve. Tissue sections of 5 µm for hematoxylin and eosin, 3µm for IHC, were acquired by utilizing automated microtome (Shandon Finesse Me +). After sectioning, the next step was to place tissues in a water bath at 45˚C temperature. Tissue sections were picked up on clean albumin coated slides. Extra water was dried off at room temperature.

Histological Techniques

Tissue staining -Hematoxylin and Eosin (H&E): The hematoxylin and eosin are most extensively used stains. Its popularity is because of its simplicity and competence to exhibit numerous cellular structures. Nucleuses of cells are stained blue black and a comprehensive intranuclear picture is given with hematoxylin. Cell cytoplasm and the majority of connective tissues are stained in range of pink orange and red shades with eosin.

Albumenized glass slide were used for tissue section for staining and histological examination and pigmented with hematoxylin and eosin (H&E) in a usual way. These were deparaffinized, hydrated by descending grades of alcohols (100%, 80%, 70%, and 50%), marked with hematoxylin and eosin (H&E) and distinguished with 0.5% acid alcohol. Later bluing in aqueous ammonia was done. Finally, the tissues slides were rinsed in water and counter stained with eosin. Subsequently these slides were dehydrated by ascending grades of alcohol (50%, 70%, 80% and 100% alcohol) cleaned in xylene and mounted in DPX (Bancroft et al., 2013)

Parameters: Vascular congestion (Present/Absent)

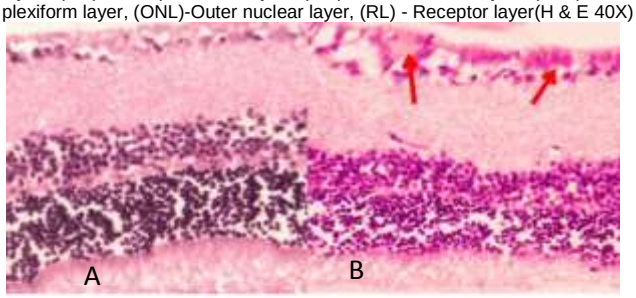
Methods for Observing Parameter:

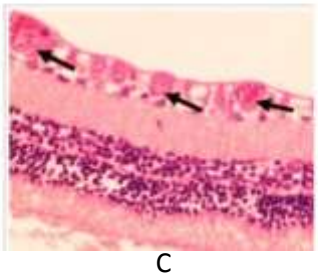
Vascular Congestion: Each tissue slide was observed at five random sites from each animal for presence (1) or absence (0) of vascular congestion. Vascular congestion was marked present when vessels were seen to be packed with blood cells. Care was taken that these sites were not overlapping with each other.

RESULTS

Thirty six complete eyeballs from total 18 animals were obtained, 12 in each group. The gross anatomy remained intact while dissection of eye ball from bony orbit. Each orbit was dissected out along with a twig of optic nerve. No shrinkage or loss of tissue during the whole process of fixing, processing, and finally paraffin embedding of eyeball was observed.

Figure 1: Histological image showing vascular congestion in RGL. **(A)** Retina of control group with no evidence of vascular congestion. **(B)** Retina of group B, vascular congestion can be appreciated in ganglion cell layer (red arrow).**(C)** Retina of experimental group C multiple vascular congestions can e observed in ganglion cell layer (black arrows). (RGL)- retinal ganglion layer, (IPL)-Inner plexiform layer, (INL)-Inner nuclear layer, (OPL)-Outer plexiform layer, (ONL)-Outer nuclear layer, (RL) - Receptor layer(H & E 40X)





Histological Examination

Vascular Congestion: In control group A no vascular congestion was observed in any specimen (Fig.1). While vascular congestion in the form of filled blood vessels was seen in the ganglion layer of group B and group C animals (Fig: 1B and 1C). In experimental group B, vascular congestion was present in 33% of animals while in group C it was 83% (Table 2). Chi^2 revealed this difference as statistically significant among the groups (p=0.006).

Table 1: Percentage distribution of vascular congestion among groups

Rats	Vascular Congestion		Total	%age vascular congestion
	Present	Absent		
Group A	*0*	*6*	*6*	*0*
Group B	*2*	*4*	*6*	*33%*
Group C	*5*	*1*	*6*	*83%*
Total	*7*	*11*	*18*	*38%*

P value 0.006

DISCUSSION

The main aim of this research project was to explore impact of carbamazepine on retinal histology of adult Wistar albino rats. To our knowledge, the observation of increased **vascular congestion** in ganglion cell layer has not been reported previously with the use of any other antiepileptic drugs. This is the first study that has noted the presence of vascular changes in the layer of retinal ganglion cells. This histological finding in retina was dose dependent. Retinotoxicity with AED use has been reported to be related with ischemic changes (Hosking et al., 2003, Hilton et al., 2002). Recent studies have related concentration of vascular endothelial growth factor VEGF with ischemia of retina (Kaur et al., 2009).This increased VEGF causes some pathological conditions, like angiogenesis, increased vascular permeability, and further inflammatory processes (Kaur et al., 2015, Joachim et al., 2017). This could explain the vascular congestion seen in our experiment.

In current study, CBZ like other AEDs might have affected ocular perfusion resulting in ischemic hypoxic insult to retina. A change in layer organization and vascular congestion (Kaur et al., 2009)can be indirectly related to ischemic changes and imbalance of neurotransmitter (Osborne et al., 2004)by CBZ. However further exploration is required to find out the exact mechanism behind these changes.

Strengths and Limitations: Our study is the first one that has reported *histological changes* by carbamazepine on retina. One of the main strengths of the study is the use of non-epileptic animals as the study population. The findings of retinal changes in these animals support the notion of CBZ affecting the retina rather than the disease itself. Another important strength is the adequate sample size, which was calculated systematically.

CONCLUSION

The results of the current study show that the carbamazepine affects retinal morphology which can lead to visual problems in patients taking medicines for prolonged period of time. Special care should be taken, and proper follow up with retinal examination is required in these epileptic patients in view of the findings of the current study.

Conflict of interest: Nothing to declare

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