



CODEN [USA]: IAJPBB

ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES<http://doi.org/10.5281/zenodo.3663201>Available online at: <http://www.iajps.com>

Research Article

**STRUCTURAL ALTERATIONS IN THE CORNEA OF RAT
AFTER MONOCULAR EYELID CLOSURE**Dr Daniyal Sajjad Awan, Dr Serwan Muttayab Mufti, Dr Haseeb Saeed
Sheikh Zayed Hospital Rahim Yar Khan**Article Received:** December 2019 **Accepted:** January 2020 **Published:** February 2020**Abstract:**

Objectives: The purpose of this study is to elaborate the histo-morphological alterations in cornea of rat after Monocular Eyelid Closure.

Methodology: In this research work, we chose 26 *Rattus norvegicus* rats of male gender having the age of 3 months. We separated the rats into three different groups; baseline (n: 2), experimental (n: 16) and control group (n: 8). we performed the monocular eyelid closure on these animals chosen for experimentation using ST (Suture Tarsorrhaphy). We euthanized 2 rats from the group of experiment and 2 rats from the group of controls on the experiment days five, ten, fifteen and twenty, we harvested their eyeballs and carried out their routine processing trichrome staining of Masson, paraffin embedding and sectioning. We took out the photo-micrographs with the utilization of the digital photo-microscope.

Results: In non-opened eyes, there was a decrease which is time-dependent during corneal epithelium's stratification with succeeding disintegration and a rise in the dissemination of stromal Keratocyte's whereas endothelial cells of cornea displayed trivial expansion from the squamous shape. The control eyes and contra-lateral eyes did not show any important alterations in the whole period of experimentation.

Conclusion: Monocular Eyelid Closure structural alterations in the epithelium of cornea, stroma and endothelium of suture tarsorrhaphy of eye. Suture Tarsorrhaphy duration cannot be increased because of the danger of the diseases of cornea and reduction of vision acuity as an outcome of the structural alterations.

KEYWORDS: Rats, Vision Acuity, Disintegration, Epithelium, Cornea, Experiment, Photo-Microscope.

Corresponding author:Dr. Daniyal Sajjad Awan,
Sheikh Zayed Hospital Rahim Yar Khan

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Please cite this article in press Daniyal Sajjad Awan et al., *Structural Alterations In The Cornea Of Rat After Monocular Eyelid Closure*, *Indo Am. J. P. Sci*, 2020; 07(02).

INTRODUCTION:

A vascularity of the cornea is very essential for the transparency of cornea [1]. But, this offers a physiological encounter for supply of different metabolic features particularly the supply of oxygen [2]. So, the supply of the oxygen by the nonvascular resources as tear film of conjunctiva, aqueous humor and direct oxygen from the atmosphere as the most essential source in the condition of open eye [3]. The closure of eyelid deprives cornea of the oxygen of atmosphere and light [4]. There are many condition for the closure of eyelid as corneal epithelial abnormalities, keratopathy, burns of chemical, neurotrophic ulcers, diabetic disease of eye, Bell's palsy and grave's disease [5]. When it is for a long duration, closure of the eyelid is a factor of risk for the anomalies like recurring erosion of cornea, de-epithelialization of cornea and keratitis which can have impacts on vision acuity [5, 6]. There is scarcity of the data on the histomorphological alterations in the corneal layers with the closure of eyelid. This research work carried out to interrogate the structural alterations in cornea following complete closure of the unilateral eyelid, utilizing rat as model.

METHODOLOGY:

As samples of this study, we used *Rattus norvegicus* male rats because the structure of the cornea in the rats shows the same histological organization in comparison with humans [7]. Additionally, this male rat specie *Rattus norvegicus* is widely in use for the eye research as model for the diseases of human eye [8]. All the samples were 3 months of age, at this age maturation of the cornea is almost complete in those animals. We used the convenient sampling to obtain the correct size of samples. We used only male rats since there are receptors for the estrogen, a factor of modulation in its bio-mechanical traits [9]. We kept the rats in cages and we regularly changed their sawdust bedding.

We used only two rats for baseline. Then, we divided the remaining 24 rats into two groups of controls and experiment. Sixteen rats were in experiment group and eight animals were in the group of healthy controls. We used the suture tarsorrhaphy for the closure of the unilateral eyelid on the animals of the experiment group, 8 rats from left and 8 rats from right eye. We anesthetized the

animals utilizing 5.0% inhalational halothane and then we made 4 horizontal mattresses on outer lamina of the margins of lower and upper eyelid utilizing polypropylene mono-filament 5.0-0 sutures. We observed the group of experiment daily to ensure the intact of the sutures. We administered the combined indomethacin eye drops 2 times on daily basis after the surgery for the prevention of the infections and reduction in pain. Rats present with decreased activity, reduced intake of water and food and enhanced aggression were further provided injection of subcutaneous ketoprofen at dose of 5.0 mg/kg. No patient No rat was present with the development of the suture dehiscence. The control rats did not experience tarsorrhaphy on any eye.

We euthanized 4 rats from the group of experiment and 2 rats from the group of controls at the experiment day five, ten, fifteen and twenty, then we harvested their eyeballs and then we processed for the sectioning and paraffin- embedding. We fixed the eyeballs of rats in 10% formalin for 12 hours. We followed it by dehydration in the increase of the alcohol grades at 1 hour intervals and toluene clearing. We embedded the eyeballs in the wax paraffin and conducted for the transverse sectioning. After cut into sections seven μm utilizing a microtome. We carried out the staining with the utilization of the method of Masson's trichrome. We used the digital camera to take the photomicrographs of all the sections. We also described the structural alterations in every layer of the cornea.

RESULTS:

Cornea contained stroma, endothelium and squamous non-keratinized epithelium for all the samples of the research work. The alterations in the membranes of Bowman and Descemet could not be obviously defined with the usage of the light microscopy. We found no observable side disparities in the corneal morphology. There were 4 to 5 layers of cells resting on the membrane of basement at baseline. Keratocyte's nuclei were present as elongated and flat. Most of the thickness of the cornea was occupied by the stroma. Simple squamous cells made the whole endothelium and nuclei of these cells were placed sparsely from each other as presented in Figure-1.

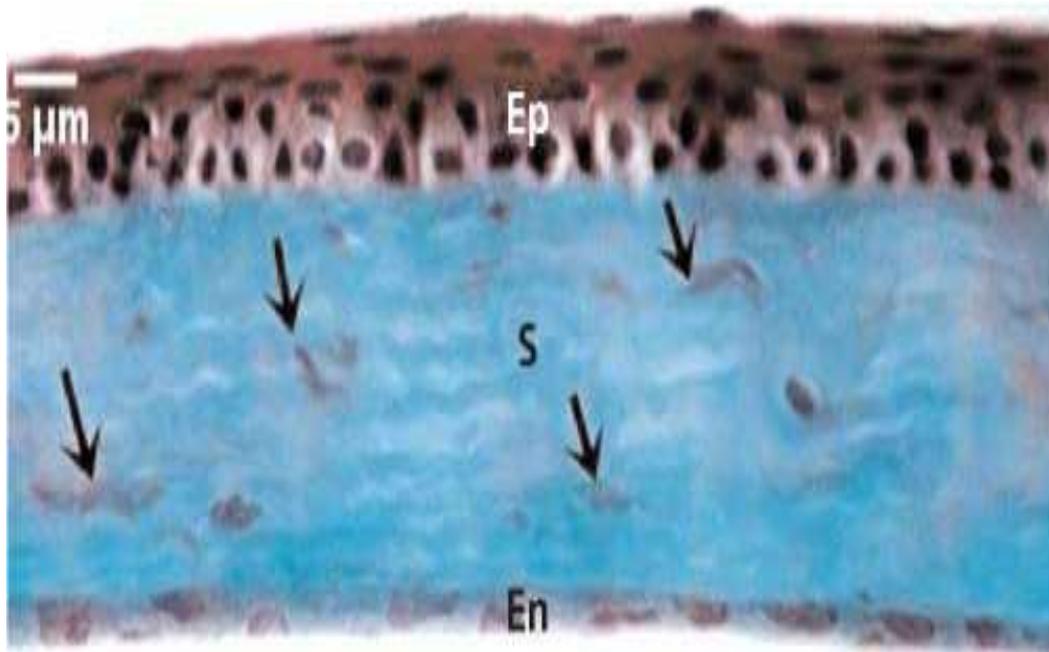


Figure 1

Table-1 provides the summary of the structural alterations as observed as compared to the members of control group.

Histological Changes In Corneal Layers With Time Of Monocular Deficiency

Corneal Layers	Tarsorrhaphy Eyes	Contralateral Open and Control Eyes
Epithelium	Stratification - Slowly decreasing (Baseline - 20th day)	Identical to Baseline
	Vacuolation and disintegration of superficial layers; basal layer intact (on 20th day)	
Stroma	Corneal Keratocyte's slowly increasing distribution observed from Baseline - day 20. Shape of Keratocyte's changed to flat from round shape (between day 15 to day 20)	Identical to Baseline
Endothelium	Endothelial cells' size was slightly increased (between day 10 - day 20)	Squamous cells, identical to Baseline

* BL : Baseline

In TE (Tarsorrhaphy Eyes), we found a gradual loss of the epithelium's superficial cells with the increase periods of monocular deprivation (Figure-2a-d).

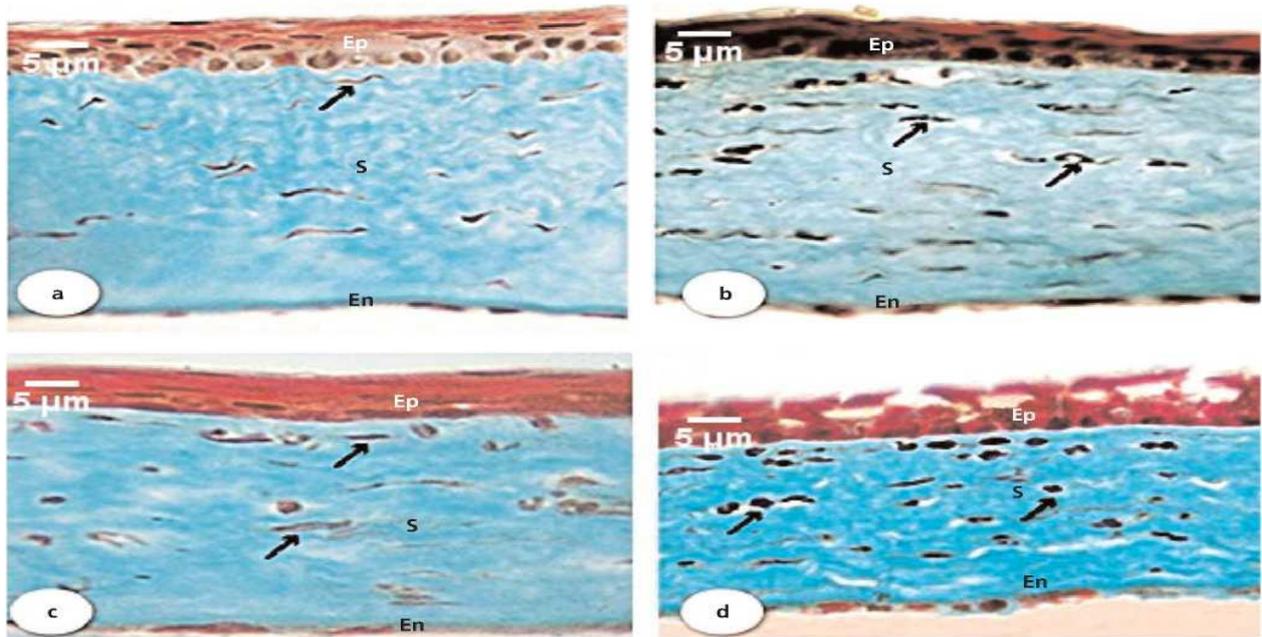


Figure 2

At experimental day twenty, we noted the disintegration and vacuolation inside the superficial, exiting only layer of base (Figure-2d). With the increase in the deprivation period, Keratocyte's inside the stroma's parallel collagen fibers enlarged in distribution (Figure-2a-d), and they were present as more rounded and elongated (Figure-2d). For all the days of research, endothelium was modest squamous for all the days but the appearance of the nuclei was slight larger from day ten to onward (Figure-2b-d). In Non-TE, the epithelium of the cornea was present as stratified squamous non-keratinized on whole days of experiments (Figure-3a-d).

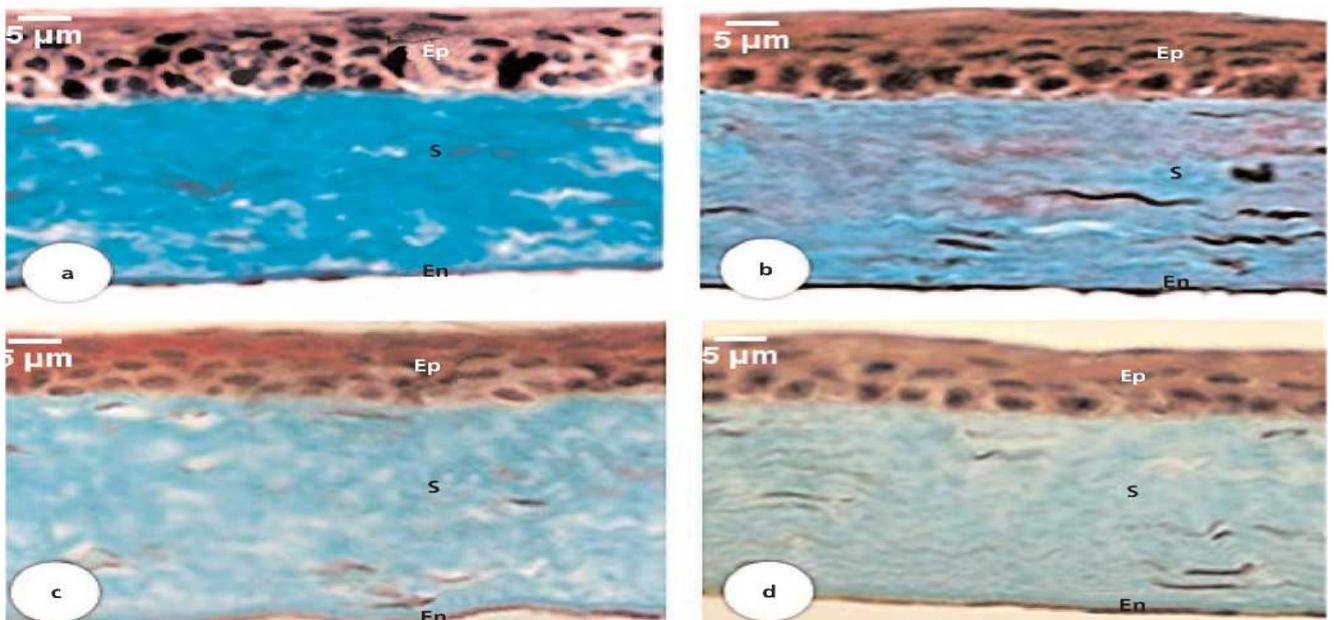
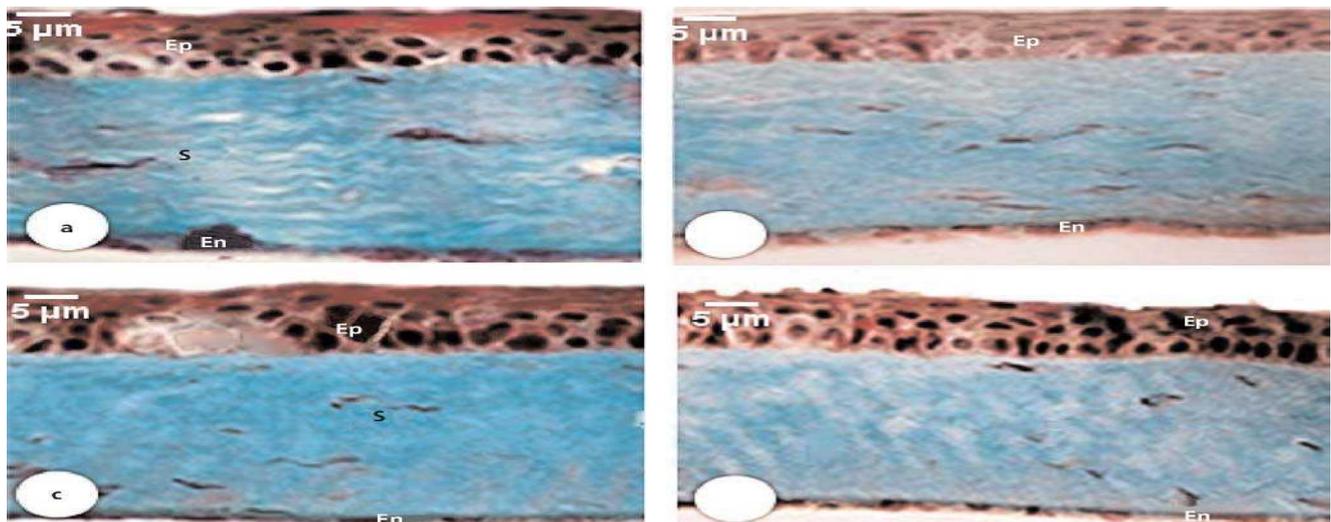


Figure 3

This was the same condition for all the eyes of controls. We observed no time-dependent alterations in the whole period of experiment (Figure-4a-d).



DISCUSSION:

Ladage in year 2003 stated a reduction in the BrdU (Bromodeoxyuridine) labelled as cells of corneal epithelium in closed eye after 8 successive days, suggesting a reduction in the proliferation with the passage of time [10]. But Yamamoto [11] carried out the closure of eyelid for twenty-four hours and stated a reduction in exfoliation in surface cell in closed eye [10]. Perez [12] suggested that levels of low oxygen suppress the process of aerobic metabolism of epithelium which have impact on the renewal of cell. The vacuolation of epithelium in the group of Tarsorrhaphy Eyes at day twenty may perhaps be an indication of the Para ptosis or Type-3 death of cytoplasmic cell [13]. This is type of the death of programmed cell that is the outcome triggered by the TNF (Tumor Necrosis Factor) or IGF (Insulin-like Growth Factor) [14]. Its characterization is possible by the reorganization of the cytoplasmic, ER and mitochondria (Endoplasmic Reticulum) enlargement, cells swelling and widespread formation of the cytoplasmic vacuoles [15]. There is portraying of the Para ptosis in the cells of the retinal ganglion succeeding ischemia due to glaucoma, which is one of the important reason behind the loss of vision in human beings [16, 17]. Additionally, Para ptosis in the epithelium of the cornea may present in pathological situations like the CD (Corneal Dystrophies) [18]. Epithelium stratification and availability of the tight junctions in combination to the complexes of other junction convene the blockade function to cornea [19]. So, any perturbation in the surface of the cornea like thinning conciliations the reliability of this particular blockade and enhances the dangers of mechanical damage as well as the microorganism's entry with the prevalence of the diseases of cornea [20].

Keratocyte's are the cells like fibroblast of stroma of cornea accountable for the repair and maintenance of the components of stroma. They usually come to perform their action when there is requirement of repair [21]. When the duration of the closure of eyelid is long, there is influence on the metabolism of cornea because of the resultant hypoxia [22]. There can be influence on the Keratocyte's by prompts from the superimposing epithelium [23]. Enhancement in the results of Keratocyte's in high synthesis of fibers of stromal collagen which can be irregular, consequential in blurred vision acuity [24, 25].

CONCLUSION:

This research work showed the degenerative alterations in the tarsorrhaphy eye that can have adverse impact in the functions of the cornea. So the findings of this research work give recommendation that there should be no increase in the tarsorrhaphy because of the dangers of the diseases of cornea and decrease of the vision acuity as an outcome of the structural alterations resulting to the decreased transparency of the cornea.

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