

HEXAVALENT CHROMIUM(VI) INDUCED TOXICITY ON RAT CORNEAL STRUCTURE: A LIGHT AND SCANNING ELECTRON MICROSCOPIC STUDY

BY

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ABSTRACT

Chromium is one of the most studied chemicals linked to occupational hazards and is a proven toxin, mutagen and carcinogen. People are exposed to chromium compound in a wide variety of situations as it is commonly used in industrial chrome plating, welding, painting, metal finishes, steel manufacturing, alloy, cast iron and wood treatment. So, this study aimed to evaluate the cytotoxicity of hexavalent chromium on rat corneas using light and scanning electron microscopic examinations. Ten adult albino rats were used as a control group, whereas twenty adult albino rats were treated with 25 and 2.5 mg/kg hexavalent chromium by intraperitoneal (ip) injection for 7 days and for 90 days respectively. Examination of the control rat cornea demonstrated that, it consisted of five layers. The outer surface was stratified squamous non-keratinized epithelium with polygonal shape and surface microvilli. After seven daily dose of 25 mg/kg hexavalent chromium administration, the examined corneal specimens revealed increased corneal thickness and loss of cellular demarcation of corneal epithelium with cleft formation and few microvilli. There were cytoplasmic vacuolation and nuclear changes. Also there was loss of the normal stromal lamellar architecture and various empty spaces. Moreover, after 90 days of chromium toxicity there was irregular corneal surface with marked patchy epithelial loss and multiple surface ostia. Also, there was morphological alteration of the surface microvilli and intercellular focal disruption. The Bowman's layer was lost. The corneal stroma showed marked loss of lamellar architecture and empty spaces. Corneal vascularization was also seen with inflammatory infiltrates. So, the present study concluded that, hexavalent chromium is very toxic to the cornea especially after prolonged exposure causing visual impairment and loss.

INTRODUCTION

Chromium is a fragile, steel grey metal which is extracted from the widespread ferruginous chromite (FeOCr_2O_3). As an

impurity; chromium is found in coal, oil, feeding stuffs, fertilizers, chalk and clay, it is used among other things in cement manufacture. Chromium combines in several oxidation phases: divalent (+II), triva-

lent (+III) and hexavalent (+VI) (Bidstrup and Wagg, 1983). It occurs in the work place predominantly in two valence states: hexavalent chromium (chromium VI) and trivalent chromium (chromium III). Hexavalent chromium compounds find extensive application in diverse industries, while trivalent chromium salts, are used as micronutrients and dietary supplements. Due to its acidic and oxidizing properties hexavalent chromium is very important in industry but it is also the most toxic and it is one of the most studied chemicals linked to occupational hazards. It is used in various alloys i.e. stainless steel and heat resistant steel, for chromium plated metals, plastics and polyethylene. Various chromium compounds are also used as dyes in car paint, textiles, glass and pottery. Chromium is widely used in leather tanning and along with arsenic and copper compounds in impregnation of wood (Bidstrup and Wagg, 1983; Bagchi et al., 2002).

There are four important routes of exposure for chromium, dermal absorption, ingestion, inhalation and ingestion secondary to inhalation. Chromium can act directly at the site of contact or be absorbed into or through human tissue. Once absorbed into the blood, it is rapidly reduced to chromium III and excreted by the kidney and liver and it is released into the urine (Ningi and Grant, 1999).

The toxic effect on skin and mucous membrane from prolonged exposure to hexavalent chromium is well known in occupational medical literature. The cardinal clinical feature is inflammation of the nasal mucous membrane with ulceration or perforation of the nasal septum. Other human health effects are papillomas in the nasal cavity, oral cavity and the larynx, lung cancer, dermatitis, kidney and liver damage (Gunaratnam and Grant, 2002).

The cornea is the clear disk-like anterior portion of the outermost fibrous supporting coat of the wall of the eye globe and lies anterior to the iris. It protrudes anteriorly and forms a small segment of the eye sphere. It constitutes the anterior one-sixth of the eyeball and is more curved than the remainder of the globe. The cornea appears slightly elliptical when viewed from in front (12 x 11mm), but it is circular when viewed from behind. The posterior surface of the cornea is more spherical than the anterior surface, so the central cornea is thinner (0.52 mm) than the periphery (0.65 mm). The zone of the transition between the cornea and sclera, known as the limbus of the cornea, is about 1mm in width. Although the sclera and cornea are structurally continuous, their line of junctions is marked externally by a slight circular furrow, the external scleral sulcus and internally by the internal scleral sulcus (Copenhaver et al., 1978; Proia, 1997). The cornea is transparent as it is devoid of blood

vessels, but it is richly supplied by sensory nerve endings via the first division of the trigeminal nerve (Copenhaver et al., 1978; Stevens and Lowe, 1997). Most of the refraction of light takes place at the surface of the cornea. Damage to the cornea may result in corneal opacity that may impair vision (Cormack, 2001).

Little data are known about hazards and biomonitoring possibilities of this type of chromium for workers who may be occupationally exposed to such compound so, the aim of this study was to evaluate the toxic effects of hexavalent chromium on the rat corneal structure using light as well as scanning electron microscopes. Also, for assessment of time-dependent effects on increase corneal damage which may help in understanding its pathogenesis.

MATERIAL AND METHODS

Chemical:

Sodium dichromate was purchased from Aldrich Chemical Co.

Animals and treatments:

Thirty (30) healthy adult white albino rats (160-180 gm) were used in this study. All animals were housed in a controlled environment at 25°C with a 12 hours light and 12 hours dark cycle and acclimated for at least 5 days before use. Animals were divided into three groups, ten for

each group: The first group was served as a control group. The second and third groups were served as experimental groups and were given a daily dose of 25 mg (0.5 LD₅₀) and 2.5mg (0.05 LD₅₀) sodium dichromate / kg body weight by intraperitoneal (IP) injection for 7 days and for 90 days respectively (Bagchi et al., 2002).

Tissue collection and preparation:

At the appropriate date of the experiment, animals were anesthetized with intraperitoneal injection of thiopental sodium and sacrificed 24 hours after the last exposure. Manual separation of the eye lids was done and a drop of the fixative was rapidly applied to each eye. Both the right and left corneas were dissected atraumatically and were resected from area behind the limbus. The right cornea of each animal was fixed in 10% formol saline as a primary fixation for at least 3-4 days and then post fixed in Bouin's solution for 24 hours only as a secondary fixation. Then it was dehydrated and embedded in paraffin. Sections were cut at 4-6 microns and stained with haematoxylin and eosin for ordinary histological examination (Drury and Wallington, 1980). The left cornea was fixed immediately in 3 % glutaraldehyde in 0.1M phosphate buffer, PH 7.4. After that, the specimens were washed in 0.1M phosphate buffer, dehydrated by ascending grades of alcohol, and critical point dried by CO₂ in Samdri-

PVT-3B-JEOL(110 volt) (Hayat, 1981; Kim et al., 2002). Hereafter, the dried samples were mounted on metal stubs, coated with gold by Ion sputtering device (JEOL JFC 1100 E) and examined by JEOL JSM-5300 scanning electron microscope.

RESULTS

Light microscopic results:

Light microscopic examination of haematoxylin-eosin stained control corneal sections showed smooth regular thickness of the cornea with five layers beginning at the outer to the inner surface. They were: a layer of stratified squamous non-keratinized epithelium, Bowman's layer, corneal stroma, Descemet's membrane and endothelium (Fig. 1). The stratified squamous non-keratinized corneal epithelium consisted of five layers. The basal cells were columnar in shape resting on basal lamina with well identified cell boundaries. Their nuclei were oval in shape with homogenous chromatin and their long axis appeared at right angles to the surface of the cornea (Figs. 1,2,3). The cells of the remaining layers were polygonal in shape in the deepest part and flat nucleated forming several layers at the surface (Fig. 2). The Bowman's layer appeared as a thin layer of acellular homogenous tissue and consisted of collagen fibrils (Fig. 3). The corneal stroma formed the bulk of the corneal thickness and consisted of connective tissue and cells, the

former was arranged in lamellae and was parallel with the corneal surface (Fig. 2). The cells of the corneal stroma (keratocytes) appeared flat parallel and were located intervening between the connective tissue lamellae (Fig.3). The Descemet's membrane appeared homogenous and was highly refractile lined by a single layer of endothelial cells which extended to form the inner epithelial lining of the cornea as a whole (Fig. 2).

Seven days after 25 mg/kg hexavalent chromium injection, the examined corneal sections revealed apparent increase of corneal thickness (Figs. 4&5) and loss of cellular demarcation of corneal epithelium (epithelial degeneration). There was a cleft between the degenerated epithelium and underlying Bowman's layer (Fig. 4). The corneal stroma showed loss of normal lamellar architecture and had empty spaces of various shape and size inbetween the connective tissue lamellae (Figs. 4&5). This was in association with presence of noticeable few corneal endothelial cells (Fig.5). Also, there was inflammatory cellular infiltration in-between the disrupted stromal lamellae (Fig. 6). On the other hand, the epithelial lining of some corneal sections appeared with loss of cell boundaries. Cytoplasmic vacuolation was also detected. Nuclear changes was marked as some of the nuclei showed margination of the chromatin especially in the superficial layer or heterogenous clumped chromatin

with irregular nuclear membrane in the deep layer (Figs. 6&7).

Ninety days after 2.5 mg/kg hexavalent chromium injection, the examined corneal sections showed apparent irregular surface and degeneration of the epithelium as there was either marked patchy epithelial loss leaving bare areas with the remaining epithelial cells appeared necrotic and had no nuclei (Figs. 8&9), or intact epithelium but exhibited loss of cellular demarcation and had no nuclei (Fig. 10). There was loss of Bowman's layer. The corneal stroma still showed loss of lamellar architecture and marked increase of empty spaces with inflammatory infiltrates (Figs. 8&10).

Scanning electron microscopic results :

The cornea of the adult control albino rat showed that the scanned surface cells were squamous and polygonal in shape. They had surface microvilli which appeared tiny and rounded. The cell borders were well defined and looked intact (Figs. 11&12).

Seven days after 25 mg/kg chromium (VI) injection, the corneal epithelium was deformed that cell configuration was hardly distinguishable due to ill defined cell borders with apparent few microvilli (Fig. 13).

Moreover, 90 days after 2.5 mg/kg chromium (VI) injection, there were multiple ostia

appeared on the surface cells (Fig. 14). Superficial vascularization of the surface of the cornea was also noticed associated with no apparent microvilli (Fig. 15). The microvilli showed different morphological alterations as, some of them were trimmed and others were absent especially around a blood vessel or around an ostium (Figs. 15&16). The cell borders showed definite intercellular focal disruption (Fig. 15). Some areas appeared with severely deformed surface epithelium and showed epithelial cell loss with disrupted cell borders so that, the surface epithelium appeared as plaques (Fig. 17).

DISCUSSION

Many chemicals found in occupational and /or environmental setting induce lesions in the mucous membrane in the form of inflammation, degeneration, and proliferation, others cause more specific effects (Shrivastava et al., 2002). Evidence of hazards was documented in studies for chromate workers (Merritt and Brown, 1995), but studies of chromium toxicity on the cornea have not widely done.

This study revealed increase of corneal thickness and evident degenerative changes as loss of cellular demarcation of corneal epithelium that cell configuration was hardly distinguishable due to ill defined cell borders with apparent few microvilli

seven days after 25 mg/kg chromium (VI) injection. Moreover, the presence of cytoplasmic vacuolation indicate occurrence of hydrobic cell degeneration. This may be due to distension of the endoplasmic reticulum and mitochondria (Cotran et al., 1999).

Proia (1997) concluded that hydropic swelling of the cells is a manifested sign of epithelial edema which in turn may result in separation of the epithelium from the Bowman's layer. The present study was consistent with this explanation as there was a cleft between the degenerated epithelium and underlying Bowman's layer which may be due to loss of intercellular attachment and cause degeneration of this layer. Unfortunately this damage does not regenerate after injury as reported by Kanski (1999) and results in the formation of an opaque corneal scar because repair is by irregular deposition of collagen (Steven and Lowe, 1997).

Nuclear changes as margination and heterogenous clumped chromatin with irregular nuclear membrane were observed. The margination of nuclear chromatin could indicate apoptotic changes as reported by Steven and Lowe (2000). But clumping of chromatin may represent one of the reactions of cells to anoxia or ischemia and is possibly due to lactate accumulation with a resulting reduction of cell pH (Trump et al., 1978; Cotran et al., 1999).

This corneal epithelial damage also may be contributed to the release of hydrolytic enzymes from the lysosomes. If this significant destruction of superficial corneal epithelium occurs together with basal epithelial cell damage, epithelial wound healing might be more compromised (Taioli et al., 1995).

With reference to the corneal stroma, there was loss of normal lamellar architecture with various empty spaces and inflammatory cellular infiltration. This may indicate occurrence of corneal edema and explained the noticeable increase of corneal thickness at this stage and results in cloudness, opacity and reduction of normal transparency of the cornea as a result of disruption of regular arrangement of corneal lamellae as reported by Kanski (1999). The above pathological changes of the cornea may impair vision.

Moreover, in this study, the noticeable decrease of endothelial cells, may give rise to fluid accumulation in the corneal stroma which may be contributed to loss of normal function of endothelium which is responsible for passage of glucose, amino acids, oxygen, salt and water from aqueous humour to the corneal stroma (Steven and Lowe, 1997). Proia (1997) accepted that the endothelium actively pumps water from the corneal stroma and is essential for normal corneal hydration. It does

not regenerate, so that loss of endothelial cells may lead to corneal edema.

The most outstanding features of 90 days of 2.5 mg/kg hexavalent chromium injection were the presence of morphologic evidence of corneal ulceration due to patchy epithelial loss and disruption of the intercellular junctions giving irregular severely deformed corneal surface. Also, degenerative changes were observed as the remaining epithelial cells appeared necrotic and had no nuclei (Copenhaver et al., 1978; Gresham, 1992). The increased empty spaces inbetween the corneal stromal lamellae may indicate increase of corneal edema which cause the overlying epithelium to separate giving epithelial ulceration with intercellular focal disruption (Steven and Lowe, 1997).

Proia (1997) reported that, chronic stromal edema leads to thinning and rupture of the epithelium with abnormal healing and deposition of basement membrane within the epithelium. As a consequence, the results of the present work were consistent with this finding as there were multiple ostia appeared on the surface cells. A similar finding was found by Kim et al. (2002) who observed exposed basement membrane at areas of detached cells in his study of 20% alcohol toxicity on rabbit corneal epithelial cells.

The surface irregularities was seem to

be from the toxic effect of chromium on superficial cells and presumably related to an abnormal epithelial wound healing process from damaged basal cells with disrupted anchoring structures (Taioli et al., 1995).

This study revealed different morphological alterations of the surface microvilli as, some of them were trimmed and others were absent especially around a blood vessel or around an ostium. This is in agreement with Kim et al. (2002) who reported deformed microvilli and disruption of intercellular junction especially with prolonged exposure and to high concentration of alcohol. Gong et al. (2003) also found decrease of corneal microvilli in magnesium deficient rats which may interfere with protection from infections, foreign bodies and dryness. Also the present work was in agreement with Van et al. (1980) who found that vitamin A deficiency lead to punctuate epithelial erosions and keratinized plaques with decreased number of microvilli on the surface of the cornea.

Moreover Johansen et al. (1994) reported a case of chronic keratoconjunctivitis with cicatricial adhesion between the tarsal conjunctiva and the bulbar conjunctiva as well as perforation of the nasal septum due to chromium vapor exposure.

Despite the fact that the cornea is nor-

mally entirely devoid of blood vessels and depends on diffusion of oxygen from the atmosphere or from the aqueous humor in the anterior chamber, corneal vascularization was detected at the surface of the examined specimens after 90 days of chromium administration which is associated with a specific corneal lesion and was reported as a major clinical problem that interfere with vision (Kanski, 1999; Proia, 1997).

With regard to the time factor, the results of the present study showed that there was more corneal damage with prolonged exposure to hexavalent chromium. This was in agreement with Kim et al. (2002) who concluded that increasing exposure time results in significant damage to superficial corneal epithelium and prolongs its normal recovery time and was also in line with Johansen et al. (1994) who concluded that chronic inflammation of the mucous membranes due to prolonged exposure to chromium may be irreversible with risk of late development of cancer.

The mechanism of cytotoxicity of chromium (VI) is not completely understood. However it can be contributed by the formation of reactive intermediates that together with oxidative stress through enhanced production of reactive oxygen species which were believed to play a key role in chromium (VI) induced carcinogenesis (Shi and Jiang, 2002). Meert et al.

(1994) suggested that immediate doses of reducing agent as ascorbic acid after ammonium dichromate poisoning would allow effective reduction of hexavalent chromium with less cellular toxicity. Moreover, Shi & Jiang (2002) had been shown that through its antioxidants properties, apple juice can protect hexavalent chromium induced cellular injury and may reduce its carcinogenic potential.

Bagchi et al. (2002) and Shrivastava et al. (2002) suggested that DNA damage, as well as enhanced excretion of urinary lipid metabolites and activation of protein kinase C, oxidative tissue damage and a cascade of cellular events including apoptotic cell death and altered gene expression may be a possible contribution of chromium toxicity.

Chromium is of significant importance in altering the immune response by immunostimulatory or immunosuppressive processes as shown by its effects on T and B lymphocytes, macrophages, cytokine production and the immune response that may induce hypersensitivity reactions (Shrivastava et al., 2002). On the other hand, Catelas et al. (2003) concluded that both cobalt and chromium ions can induce macrophage mortality in a dose-and time-dependent manner with initial apoptosis followed by necrosis. The results of the present work were consistent with the above findings as most of the nuclear

changes showed either apoptotic or necrotic changes. These results indicated evidence of two different pathways (apoptosis or necrosis) as interpretation of chromium toxicity (Schmitz et al., 2003).

CONCLUSION

It could be concluded that toxicity by hexavalent chromium resulted in definite structural damage of the cornea of the albino rat that may lead to visual impairment.

RECOMMENDATIONS

Protective equipment in the form of breathing masks and protective eye glasses should be used in this type of work unless moistening or a surface finish on the chromium sheeting is capable of preventing the release of dust. The introduction of cytological nasal examination in health surveillance programs for this category of workers acquires considerable importance. Also, regular examination for excluding malignancy must be done.

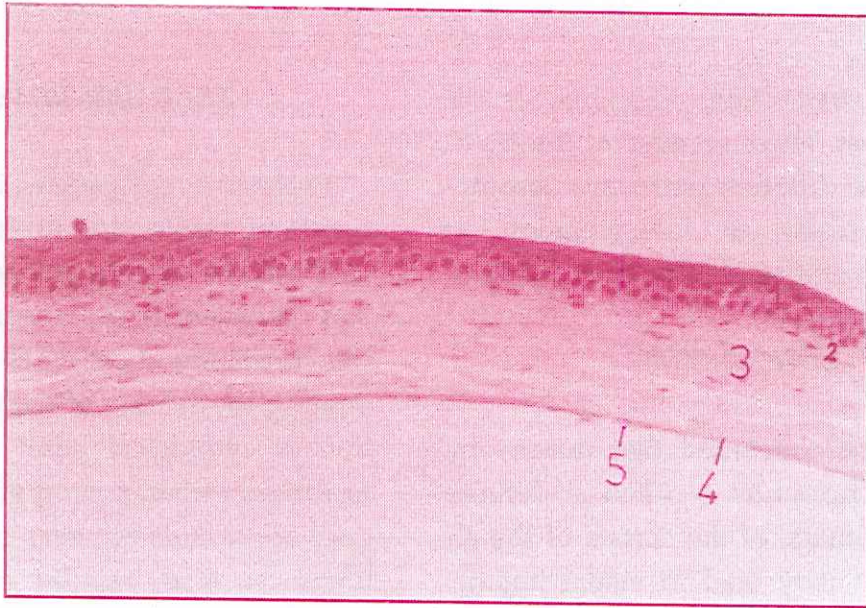


Fig. (1) : A photomicrograph of a section through the cornea of the eye of adult control albino rat showing that the cornea has regular thickness with smooth surface and consists of five distinct layers from outer to inner: 1-stratified squamous non-keratinized epithelium (5 layers of cells), 2- Bowman's layer, 3-corneal stroma, 4- Descemet's membrane and 5-endothelium. (Hx & E X200)

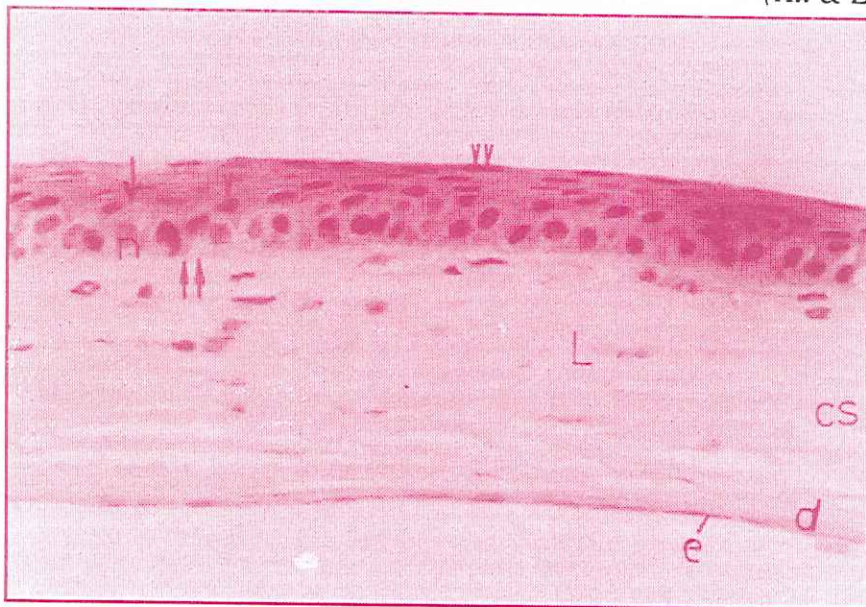


Fig. (2) : A magnified photomicrograph of the previous section showing the basal cells of the external stratified squamous non-keratinized corneal epithelium appear columnar in shape (arrow) resting on basal lamina (double arrow) with well identified cell boundaries and have oval nuclei (n). Above the basal cells there is a layer of polygonal cells (arrow head). The most superficial cells appear flat nucleated forming several layers (double arrow head). The connective tissue lamellae (L) of the corneal stroma (cs) appears parallel with the corneal surface and forms the bulk of the corneal thickness. The Descemet's membrane (d) appears homogenous in shape and lined by a single layer of endothelial cells (e). (Hx & E X400)



Fig. (3) : A magnified photomicrograph of another area of the same section showing the nuclei of the basal layer of the corneal epithelium are oval in shape with homogeneous chromatin (arrow head) and their long axis appears at right angles to the surface of the cornea. Bowman's layer (*) appears as a thin layer of acellular homogenous tissue. The stromal cells (keratocytes) are flat (arrow) embedded in-between the connective tissue lamellae. (Hx & E X1000)

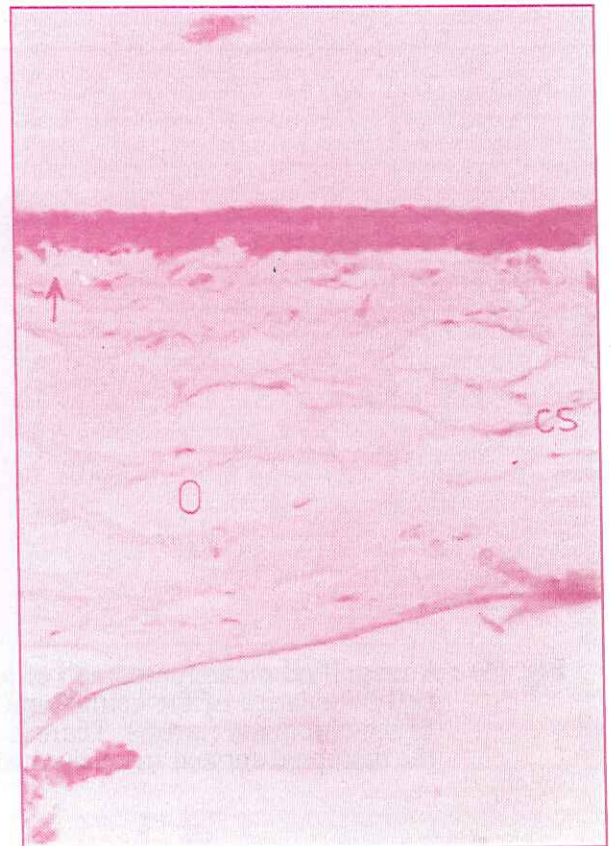


Fig. (4): A photomicrograph of a section of the cornea of albino rat 7 days after hexavalent chromium injection showing apparent increase of corneal thickness and loss of cellular demarcation of corneal epithelium (*). There was a cleft (arrow) between the degenerated epithelium and the underlying Bowman's layer. The corneal stroma (cs) appears with loss of normal lamellar architecture and has empty spaces (O) of various shape and size. (Hx & E X200)

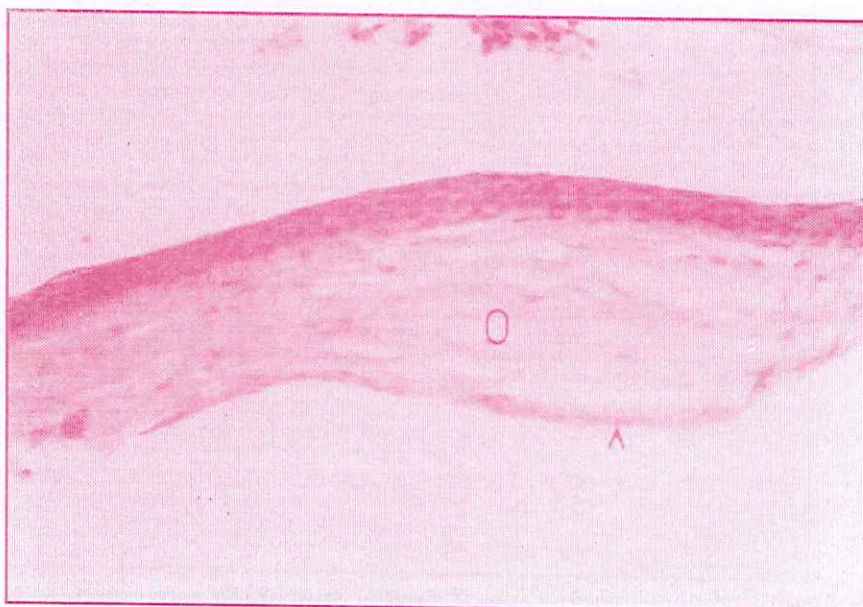


Fig. (5): A photomicrograph of another corneal section of albino rat after 7 days hexavalent chromium injection demonstrating noticeable increase of corneal thickness with empty spaces (O) and apparent few corneal endothelial cells (arrow head).
(Hx & E X200)

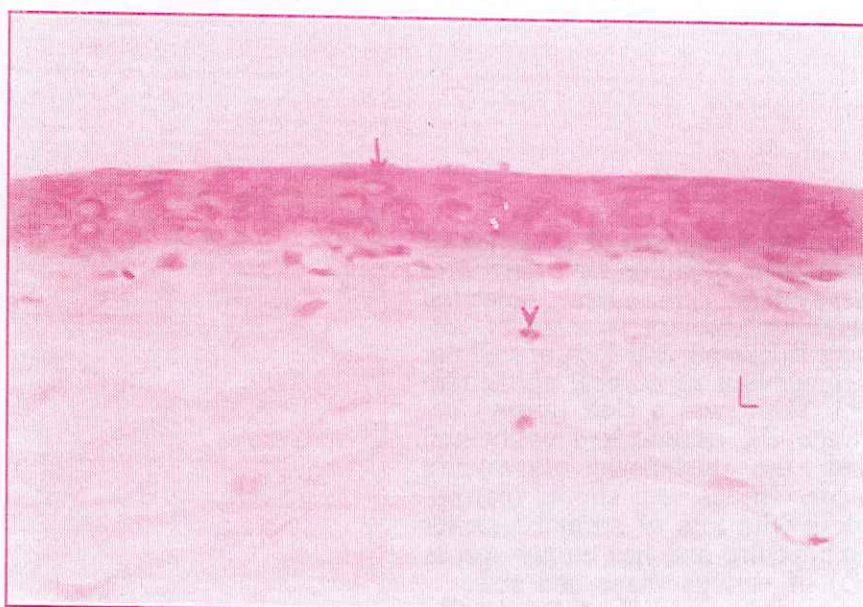


Fig. (6) : A magnified photomicrograph of another area of the same section showing loss of cell boundaries of the epithelium(*). Most of the nuclei appear with margination of the chromatin (arrow). There is inflammatory infiltration (arrow head) invading the disrupted corneal stromal lamellae (L).
(Hx & E X400)

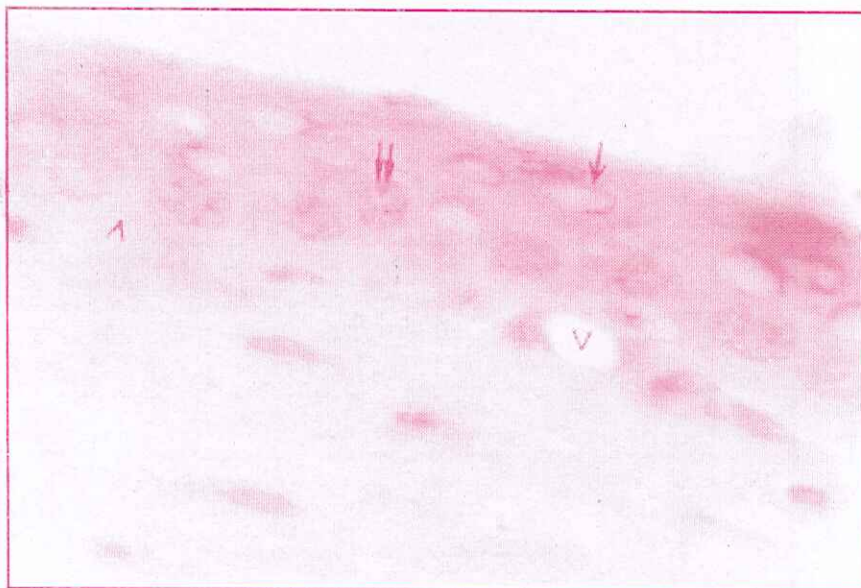


Fig. (7) : Another magnified photomicrograph of the previous section showing a large cytoplasmic vacuole (v) in the epithelium. The basal lamina appears ill-defined (arrow head). The nuclei have either marginated chromatin (arrow) especially in the superficial layer of the epithelium or heterogenous clumped chromatin (double arrow) with irregular nuclear membrane. (Hx & E X1000)

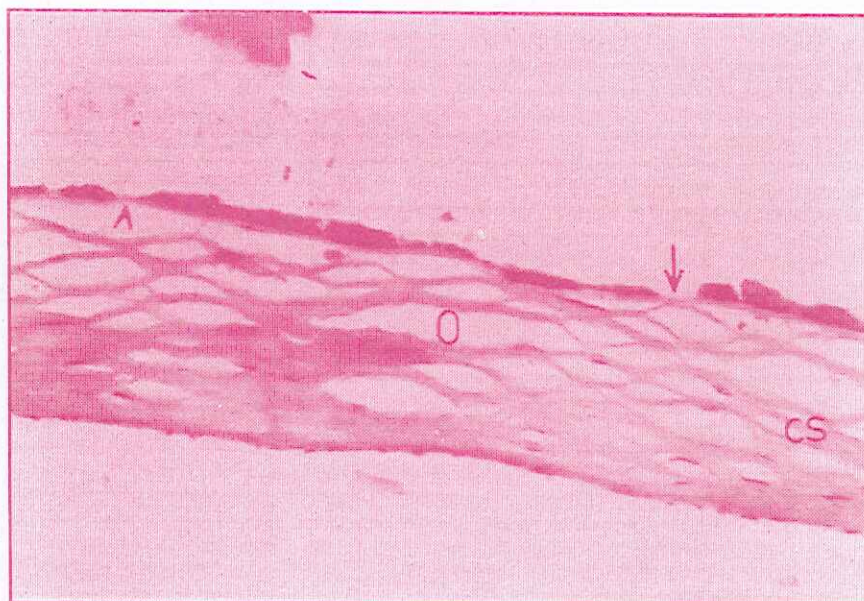


Fig. (8) : A photomicrograph of a section of the cornea after 90 days of hexavalent chromium injection illustrating an area with irregular surface and marked patchy epithelial loss (arrow) reaching the basal lamina associated with loss of Bowman's layer (arrow head). The corneal stroma (cs) still shows loss of lamellar architecture and there is marked increase of empty spaces (O). (Hx & E X200)

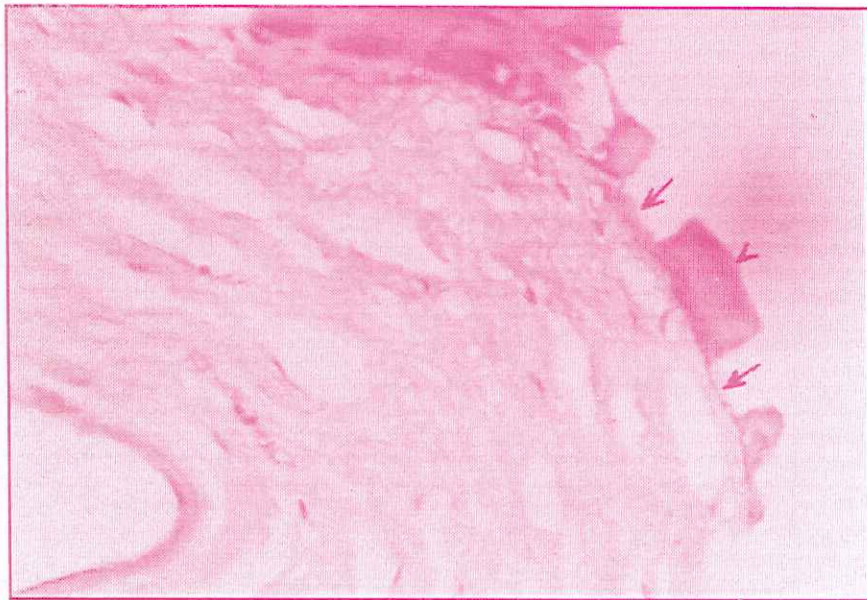


Fig. (9) : A photomicrograph of a section of the cornea after 90 days of hexavalent chromium injection showing epithelial cell loss leaving bare areas (arrows). The remaining epithelial cells appear necrotic and have no nuclei (arrow head).
(Hx & E X400)

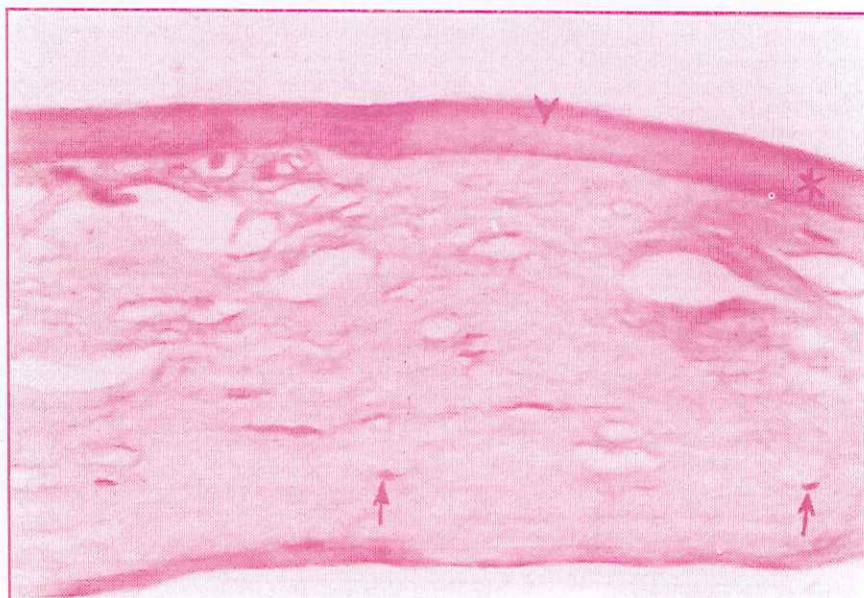


Fig. (10) : A photomicrograph of a section of the cornea after 90 days of hexavalent chromium injection showing another area with loss of cellular demarcation of the epithelial cells (*) and exhibits loss of their nuclei (arrow head). The underlying stroma shows inflammatory infiltration (arrows). (Hx & E X400)

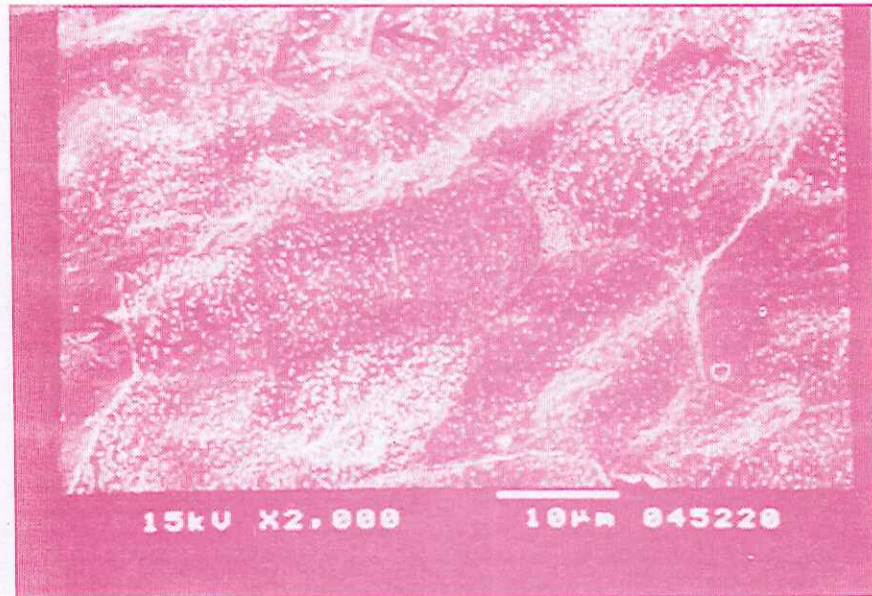


Fig. (11) : A scanning electron micrograph of the surface of the cornea of adult control albino rat showing squamous corneal epithelium, its cells appear polygonal in shape and having well defined cell borders (arrows) and surface microvilli. (X 2000)



Fig. (12): A scanning electron micrograph of the surface microvilli which appear tiny and rounded (arrows). Three polygonal surface cells also can be seen with well defined intact cell borders (double arrows). (X 10000)



Fig. (13): A scanning electron micrograph of the surface of the cornea of albino rat 7 days after hexavalent chromium injection showing an area with ill defined cell borders and hardly distinguishable cell configuration associated also with apparent few microvilli (*). (X2000)



Fig. (14): A scanning electron micrograph of the surface of the cornea of albino rat 90 days after hexavalent chromium injection demonstrating multiple ostia (arrows) on the surface cells. (X1000)



Fig. (15): A scanning electron micrograph of the surface of the cornea of albino rat 90 days after hexavalent chromium injection showing definite intercellular focal disruption of cell borders (arrows). Note, a blood vessel (double arrow) appears on the surface of an area with no apparent microvilli (arrow heads). (X1500)

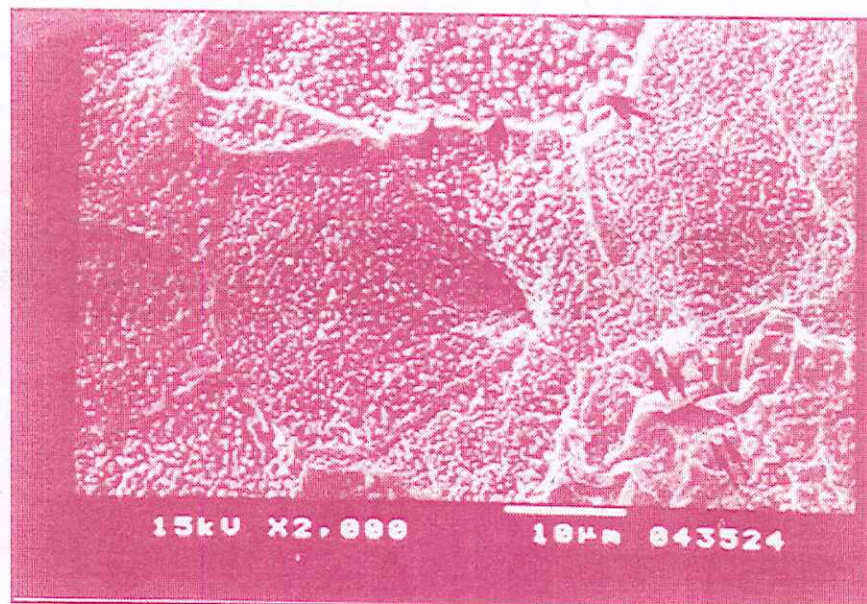


Fig. (16): A scanning electron micrograph of a portion of the surface of the cornea of albino rat 90 days after hexavalent chromium injection illustrating that the surface microvilli appear with different shapes, some are trimmed (arrows) and others can not be detected (absent) (double arrows) around an ostium. (X 2000)



Fig. (17): A scanning electron micrograph of another portion of the surface of the cornea of albino rat 90 days after hexavalent chromium injection showing severely deformed surface epithelium with definite epithelial cell loss (*) and disrupted cell borders (arrows) so that the corneal surface appears as plaques. (X 2000)

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التأثير السام للكروم السداسى التكافؤ على بنية قرنية عين الفأر : دراسة بالمجهر الضوئى والمجهر الإلكتروني الماسح

المشركون فى البحث

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توجد مادة الكروم فى صورتين من حيث قوة التكافؤ، الكروم السداسى والكروم الثلاثى ويستخدم الكروم السداسى التكافؤ فى الصناعة مثل طلاء المعادن واللحام والدهانات وصقل المعادن وصناعة الصلب والإشابة وسبيكة حديد الزهر ومعالجة الخشب وهو معروف بأنه سام كما أنه من المواد المسرطنة.

ولهذا تهدف هذه الدراسة إلى تقييم تأثير مادة الكروم السداسى التكافؤ على قرنية العين للفأر الأبيض وذلك بالفحص بالمجهر الضوئى والمجهر الإلكتروني الماسح.

وقد أجرى هذا البحث باستخدام عشرة من الفئران البيضاء البالغة كمجموعة ضابطة كما تم حقن عشرين فأراً ٢٥ و ٢٥ مع/كم من الكروم السداسى التكافؤ عن طريق الغشاء البريتونى كلا على حدة لمدة ٧ أيام ولمدة ٩٠ يوم على التوالى.

وقد أوضحت نتائج هذه الدراسة فى المجموعة الضابطة أن لقرنية العين فى الفأر الأبيض سطح أملس وهى تتكون من خمس طبقات وهم طبقة طلائية طبقية توسفية) تقشرية (غير كيراتينية وهى أيضاً تتكون من خمس طبقات كما يظهر سطح هذه الطبقة على هيئة شكل هندسى مغطى بخمليات. وتلى هذه الطبقة طبقة باومان ثم النسيج الأساسى للقرنية وهى تمثل معظم سمك القرنية وتغطى هذه الطبقة من الداخل بغشاء ديسمت يليها الغشاء البطانى.

وبعد الحقن بمادة الكروم لمدة إسبوع وجد زيادة فى سمك القرنية وعدم وضوح حدود الخلايا مصاحب بقلة الخمليات وتغيرات فى كروماتين النواة مع وجود تحويقات فى السيتوبلازم وظهور شق بين الطبقة الأولى والثانية أما النسيج الأساسى فقد لوحظ فيه فراغات متنوعة كتورمات مائية مصحوبة بارتشاح إنتهابى وبقلة ملحوظة فى خلايا الغشاء البطانى.

وقد إمتد هذا التأثير أيضاً بوضوح بعد تسعين يوماً من الحقن حيث وجدت تغيرات جسيمة من تعرجات فى سطح القرنية وتغيرات إنحلالية مع ظهور تقرحات. كما إزدادت الفراغات فى النسيج الأساسى. وقد أوضح سطح القرنية تغير متنوع فى شكل الخمليات وشقوق بين الخلايا وظهور أوعية دموية. ومن هذه النتائج تبين حدوث تغير فى الهيكل العام للقرنية ويزداد هذا التغير بزيادة الوقت.

ولهذا فإن هذه الدراسة توضح أن التعرض الوظيفى لمادة الكروم السداسى التكافؤ لفترات مختلفة يحدث تغير فى بناء القرنية مما قد يؤدي إلى فقدان شفافيته وتورم مائى وعتمة مما يزيد من مخاطر فقدان وظيفة القرنية كعضو مهم للإبصار.