

Histological changes in the Lacrimal gland of Rabbits after exposure to Formalin and treatment by Ciprofloxacin

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Keywords: lacrimal gland, Rabbits, Formalin, Ciprofloxacin.

Abstract:

The present study was designed to describe the histological changes in the lacrimal gland of adult rabbits after exposure to formalin and treated by ciprofloxacin. Lacrimal gland samples of fifteen rabbits were collected and divided into three groups, group (I) included (5) rabbits represented control group, while the rabbits in groups (II) included (5) rabbits exposed to formalin, were given two drops were instilled in the one eye/ day of formalin 10% for (7) days. Group (III) which included (5) rabbits were given two drops / day of formalin 10 % were instilled in the one eye for (7) days and in eighth day were treated with eye drops of 0.3% ciprofloxacin. The ciprofloxacin eye drops were instilled in the one eye of each rabbit for (5) days. The samples were fixed in 10 % formalin, routine histological technique was done and stained with Hematoxylin and Eosin (H& E). The present study revealed as a result of exposure to formalin, there were hypertrophy and hyperplasia in the acinar epithelial cells, increase in the secretory activity of acinar cells, destroyed in some acini also was seen, marked dilation and congestion in the blood vessels and clear dilation in the interlobular ducts. after treatment by ciprofloxacin in group (III), the histological observation of lacrimal gland was similar to that in (I) group.

التغيرات النسجية في الغدة الدمعية للأرانب بعد تعرضها للفورمالين ومعالجتها بالسبيروفلوكساسين

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الكلمات المفتاحية: الغدة الدمعية، الارانب، فورمالين، سايبروفلوكساسين
الخلاصة

صممت الدراسة الحالية لوصف التغيرات النسجية في الغدة الدمعية للأرانب البالغة بعد تعرضها للفورمالين ومعالجتها بالسبيروفلوكساسين. جمعت عينات الغدد الدمعية لخمس عشرة ارنب وقسمت الى ثلاث مجاميع. المجموعة الاولى بعدد خمسة ارانب تمثل مجموعة السيطرة، المجموعة الثانية بعدد (5) ارانب تمثل مجموعة الارانب التي عرضت للفورمالين، حيث تم اعطاء الفورمالين بشكل قطرات بتركيز 10% وبواقع قطرتين في العين يوميا ولمدة (7) ايام. المجموعة الثالثة بعدد (5) ارانب والتي عرضت للفورمالين بشكل قطرات بتركيز 10% وبواقع قطرتين في العين يوميا ولمدة (7) ايام وفي اليوم الثامن عولجت بالسايروفلوكساسين بتركيز 0.3 % وبشكل قطرات لمدة خمسة ايام. ثبتت العينات بمحلول الفورمالين وبتركيز 10% واجريت عليها التحضيرات النسجية الروتينية وصبغت بصبغة الهيماتوكسيلين والايوسين. اظهرت النتائج ان التعرض للفورمالين سبب تغيرات نسجية في الغدة الدمعية حيث لوحظ حدوث زيادة في عدد وحجم الخلايا السنخية وزيادة في الفعالية الافرازية لهذه الخلايا وحدث تحطم في بعض الاسناخ كما لوحظ توسع واحتقان في الاوعية الدموية وتوسع في القنوات بين الفصوص للغدة. كما

اظهرت النتائج ان التركيب النسيجي للغدة بعد المعالجة بالسايبروفلوكساسين كان مشابها للتركيب النسيجي للغدة في مجموعة السيطرة.

1. Introduction

Formalin is colorless fluid and contains 37% of formaldehyde and is important in medical education as preservative, disinfectant and embalming agent [1]. Most of the students in the anatomist and medical school dissection exposure to formaldehyde. Also the workers in the laboratory use the formalin for the histological and pathological fixation of the tissues, so that the direct exposure to formaldehyde vapor may cause burning sensation to the eye, nose and lung causing rhinorrhea and watery discharge from eye [2].

Lacrimal gland is Irregular round to oval gland, white to pale brown in color, located in the dorsolateral aspect of the orbit [3]. Histologically, the Lacrimal gland is exocrine compound tubuloacinar gland, consist of several lobes that divided into numerous lobules separated by connective tissue. Every lobule consist of secretory units called acini, the acini secretes its products to duct system [4,5].

The acinar cells of the lacrimal gland In the rabbit lined with pyramid cells, the epithelial cells of the ductal system changes from the simple layer of cuboidal cells in the intercalated ducts to columnar, pseudostratified and stratified columnar in the collecting duct [6].

Lacrimal gland responsible for produce the lacrimal fluid (tears), the lacrimal fluids contain proteins and electrolytes which responsible for the nourish, lubricate and moister the cornea and pupil and protect the eye from the pathogen [7].

Ciprofloxacin is a flouroquinolone antibiotic and its active drug for wide variety of gram negative and gram positive bacteria. It's a very important antibiotic in ophthalmology because it's one of only a little antibiotics which ambulate from the digestive system to the eye after oral administration[8].

Ciprofloxacin may play an active role in protect the eye from the inflammation which may occur after surgical endophalamitis because the ability of Ciprofloxacin to enter the surgical site in concentrations that reduce the bacterial count [9].

Most research that discussed the effect of formalin has focused on the effect of formalin on the respiratory system, digestive system and skin while the effect of formalin on the histological structure of the lacrimal gland was very rare.

The aim of present study is to focus the light on the histological changes in the lacrimal gland due to exposure to formalin and after treatment with ciprofloxacin.

2. Materials and Methods

Fifteen healthy adult male rabbits were obtained from the national center for drug control and research were used, they were kept under the same environmental conditions. The animals were kept in stainless steel mesh cages, the temperature was maintained at (22-27) C°, a dry, absorbent, bedding materials, like wood shaving were provided in all cages. All animals were allowed free access to food and water. All the animals were observed for (7) days before the beginning of the experiment to exclude any possibility of abnormal behavior and disease. The animals were divided into three groups as following.

- 1- Group (I) which included (5) rabbits were given two drops were instilled in the one eye/ day of normal saline 0.9 % for (7) days.
- 2- Group (II) Which included (5) rabbits were given two drops were instilled in the one eye/ day of formalin 10% for (7) days.
- 3- Group (III) which included 5 rabbits were given two drops/ day of formalin 10 % were instilled in one eye for (7) days and in eighth day were treated with eye drops of 0.3% ciprofloxacin. The ciprofloxacin eye drops were instilled in the one eye of each rabbit for (5)

days as per the following schedule. Day 1: (2) drops every (30) minutes for first (2) hours, then (2) drops every (4) hours (total of (8) doses per day), Day 2-5: (2) drops every (2) hours (total of (6) doses) [10].

At the end of the experiment, the animals were anesthetized using combination of diazepam (5) mg/kg and ketamine (25) mg/kg of body weight intramuscularly. Tissue samples were taken from lacrimal gland, The samples were fixed in 10 % formalin, routine histological technique was done and stained with Hematoxylin and Eosin (H & E) [11]. The prepared slides were examined in light microscope (Motic, Malaysia). The slides were imaged by using digital camera (14.1) megapixel power resolution camera.

2.1. Statistical analysis:

One way ANOVA was used to estimate mean \pm SE of the data and the variance was analyzed, Post hoc test was used LSD to specify the significant difference among means the software package IBM SPSS Program version 20 was used for the analysis of data [12].

3. Results

In group (I), no histological changes were seen, the lacrimal gland was consist of several lobes separated by strands of connective tissue, each lobe divided into several lobules, each lobule consisted of groups of acini in different shape and size (Figure 1). Each acini lined with simple cuboidal epithelium with spherical centrally located nucleus, each acini surrounded by numerous myoepithelial cells, little number of acini contained secretion in their lumens (Figure 2)

The present study revealed as a result of exposure to formalin, there were hypertrophy and hyperplasia in the acinar epithelial cells so that the acinar epithelial cells were high cuboidal to columnar with near basement membrane nucleus (Figure 3), so that height and number of acinar epithelial cells were significantly increase ($P < 0,05$) in group (II) as compared to (I) and (III) groups (Table 1).

In group (II), The diameter of acini was significantly larger ($P < 0,05$) than that in (II) and (III) groups (Table 1) with the presence of high secretory activity in there lumens (Figure 4, 5) and The secretory activity was very prominent in the acinar cells represented by presence of many secretory vesicles in their cytoplasm (Figure 5). As a result to effect of formalin in group (II) some acini destroyed so that there were cells infiltration (Figure 6, 7).

The present study revealed there were a marked dilation and congestion in the blood vessels in group (II) (Figure 7) as compared to group (I) and (III) with clear dilation in the interlobular ducts with marked infiltration of cells and accumulation of secretion in their lumens (Figure 7, 8).

After treatment by ciprofloxacin in group (III), the histological observation of lacrimal gland was similar to that in group (I) so that the acinar epithelial cells were simple low cuboidal with centrally located nucleus (Figure 9) and there were no significant differences between group (III) and group (I) in high and number of acinar epithelial cells (Table 1), also there were no significant differences in diameter of acini and interlobular duct (Table 1).

4. Discussion

The present study revealed that exposure to formalin had an irritating effect on the lacrimal gland of the rabbit. The histological changes in the lacrimal gland after exposure to formalin as

hyperplasia and hypertrophy in the glandular epithelium which represented by a significant increase ($P < 0,05$) in number and height of acinar epithelial cells are consistent with a number of previous studies about the effect of formalin on the respiratory system [13, 14 and 15] and on the skin [15].

Also the acinar diameter increased after exposure to formalin in the present study, Similarly [15] showed a clear increasing in the diameter of tracheal gland as a consequence to increasing in the number of glandular epithelium.

[16, 17] suggested that the formaldehyde has a carcinogenic potential after repeated exposure on the respiratory epithelium and appearance of multiple mitotic figures indicated formaldehyde carcinogenicity. [18] mentioned that formaldehyde is carcinogenic at the site of contact as a result of epithelial cell regenerative proliferation resulting from cytotoxicity and mutation.

Secretory activity was very high in the group (II) as compared to (I) and (III) groups represented by presence of many acini filled with secretion as well as presence of secretory vesicles in acinar epithelial cells. Similar finding was noted by [19] when mentioned the presence of more mucoid secretion in the lumen of nasal glands due to irritation effect of formaldehyde. Also [20] reported increase the secretory activity from the lacrimal gland after exposure to formalin and the irritating effect of formalin was reported by studying acute toxic effects of formalin-treated cadaver, medical students reported symptoms such as itching in the eyes, excessive lacrimation and redness in the eyes.

In the present study, effect of formalin on the blood vessels was conspicuous, blood vessels were dilated and congested after exposure to formalin. This result was consistent with the result of [21] when showed congestion and hemorrhages in the respiratory organs of rabbit after exposure to formaldehyde vapor.

Present study showed that some acini destroyed as a consequence to irritant effect of formalin, this result in agreement with result of [15] when they reported a destruction of alveolar septa in the rabbit lung when exposed to formalin.

after treatment by ciprofloxacin in group (III), the histological observation of lacrimal gland was similar to that in group (I), this result reflect the ability of ciprofloxacin to repair the tissue of the eye as reported by [10] when mentioned that the use of ciprofloxacin is common to treat the severe and destructive inflammation, occurring during infection of the eye.

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Table (1): showing averages of number, height of acinar epithelium and diameter of acini and interlobular ducts of lacrimal gland in rabbit.

Parameter Group	Diameter of acini (μm^2) Mean \pm S. E	No. of acinar epithelium Mean \pm S. E	Height of acinar epithelium (μm) Mean \pm S. E	Diameter of interlobular duct (μm^2) Mean \pm S. E
I	72.5 \pm 2.12 a	23 \pm 0.64 a	8.4 \pm 0.23 a	88.6 \pm 2.27 a
II	133.8 \pm 1.54 b	38 \pm 1.59 b	16.2 \pm 1.18 b	175 \pm 4.11 b
III	81.6 \pm 0.82 a	26 \pm 0.44 a	7.5 \pm 0.66 a	102.3 \pm 1.37 a

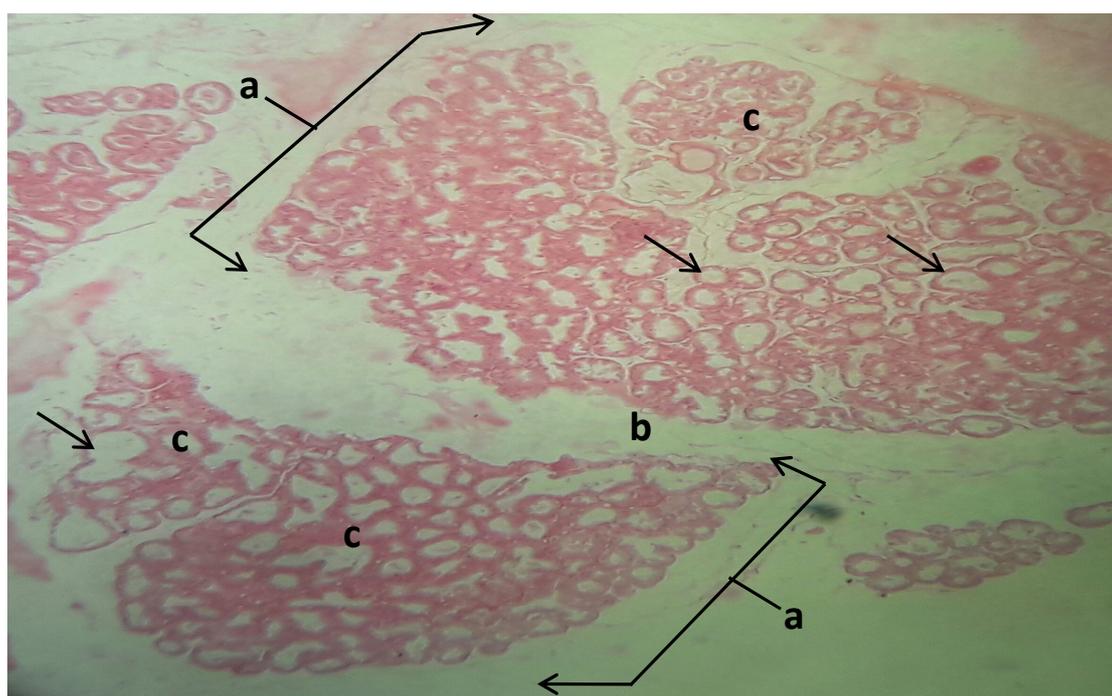


Fig. (1): Histological section of lacrimal gland in rabbit in group (I) consisted of several lobes (a) separated by strands of connective tissue (b). each lobe divided into several lobules (c), each lobule consisted of groups of acini in different shape and size (arrows). H & E stain (X 40).

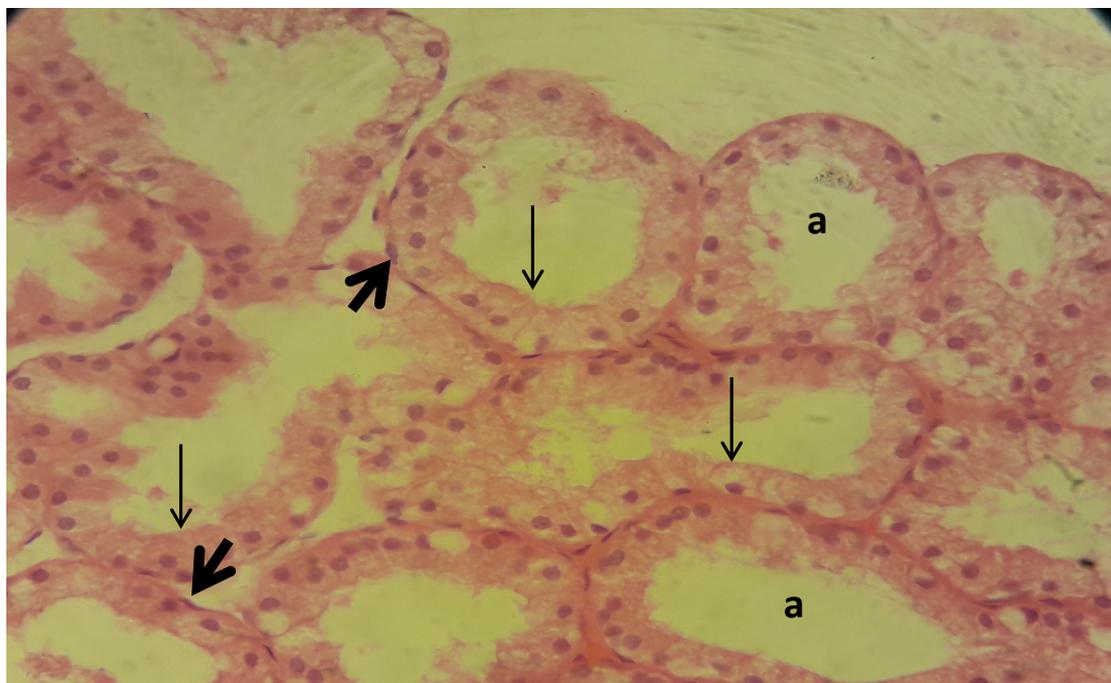


Fig. (2): Histological section of lacrimal gland in rabbit in group (I) showing acini (a) lined with simple cuboidal epithelium (thin arrows), each acini surrounded by myoepithelial cells (thick arrows) contained secretion in their lumens. H & E stain (X 400).

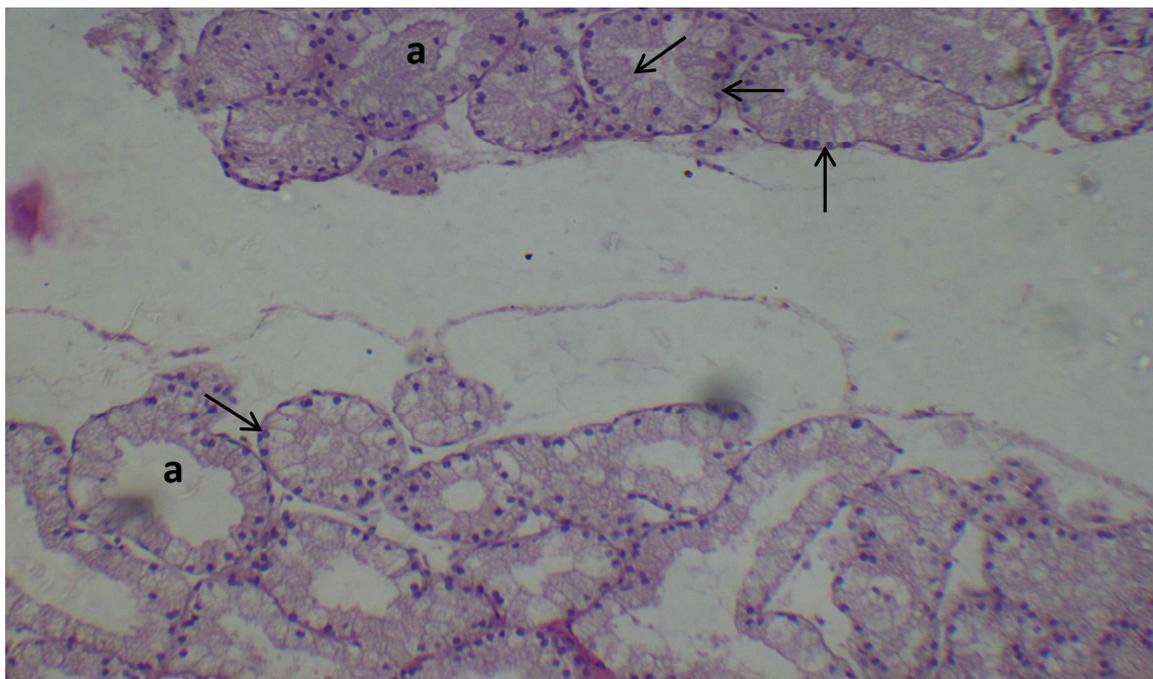


Fig. (3): Histological section of lacrimal gland in rabbit in group (II) showing acini (a) lined with simple high cuboidal to columnar epithelium (arrows). H & E stain(X 100).

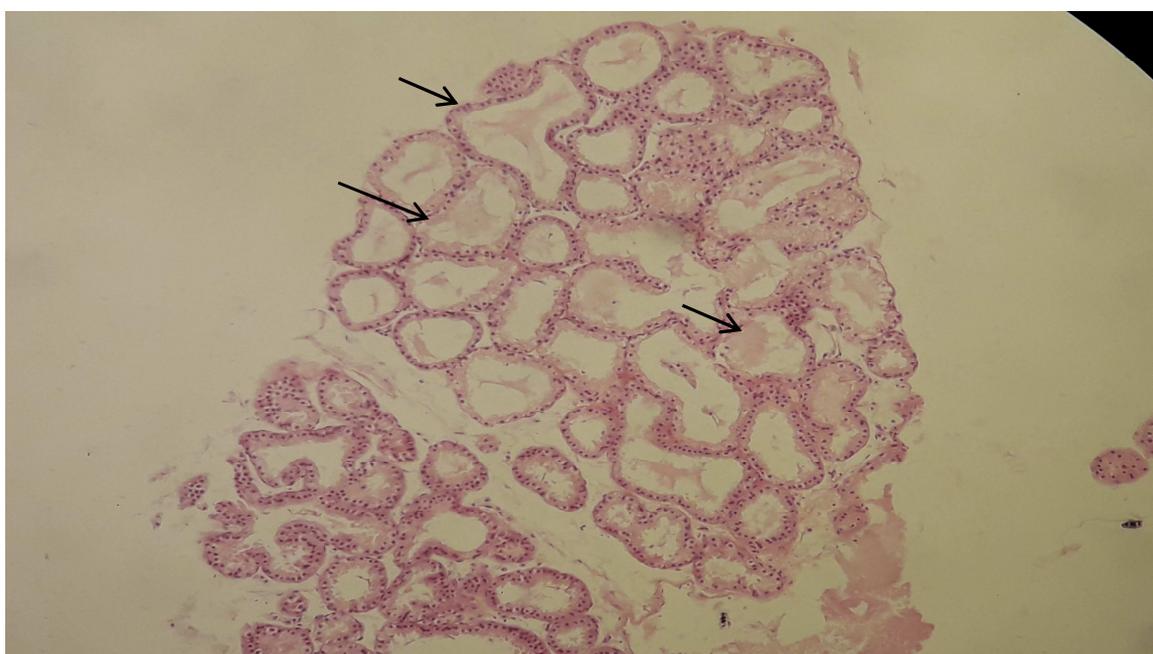


Fig. (4): Histological section of lacrimal gland in rabbit in group (II) showing acini (arrows) with its secretory activity. H & E stain (X 40).



Fig. (5): Histological section of lacrimal gland in rabbit in group (II) showing acini (arrows) with its secretory activity with presence of many secretory vesicles in their cytoplasm. H & E stain (X 1000).

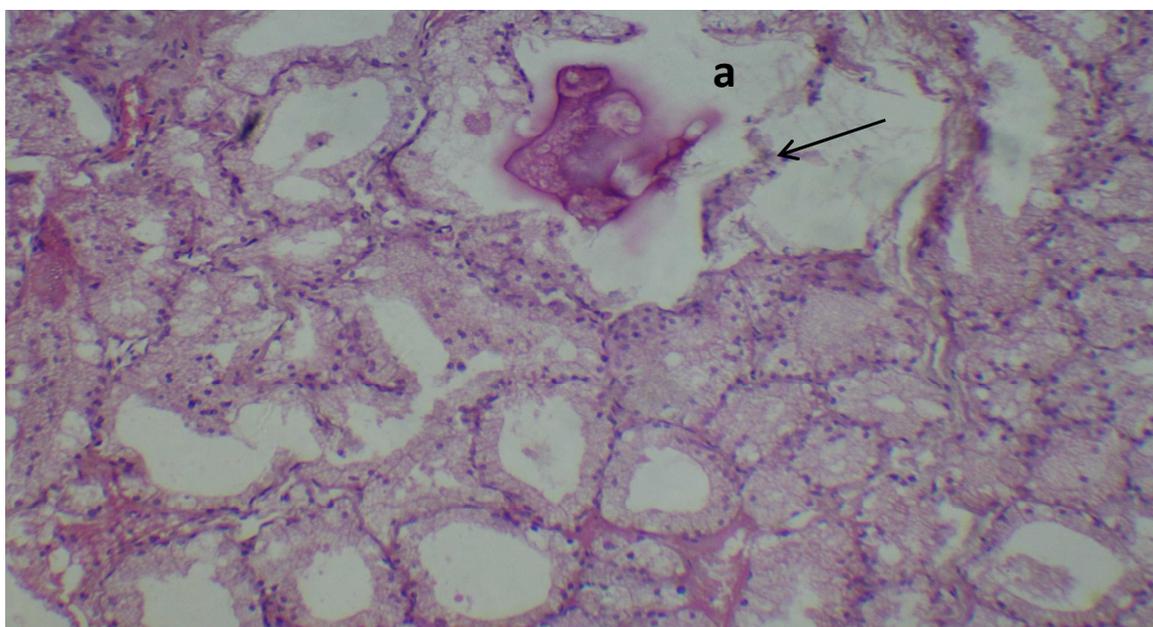


Fig. (6): Histological section of lacrimal gland in rabbit in group (II) showing some acini destroyed (a) with cells infiltration in their lumen (arrows). H & E stain (X 400).

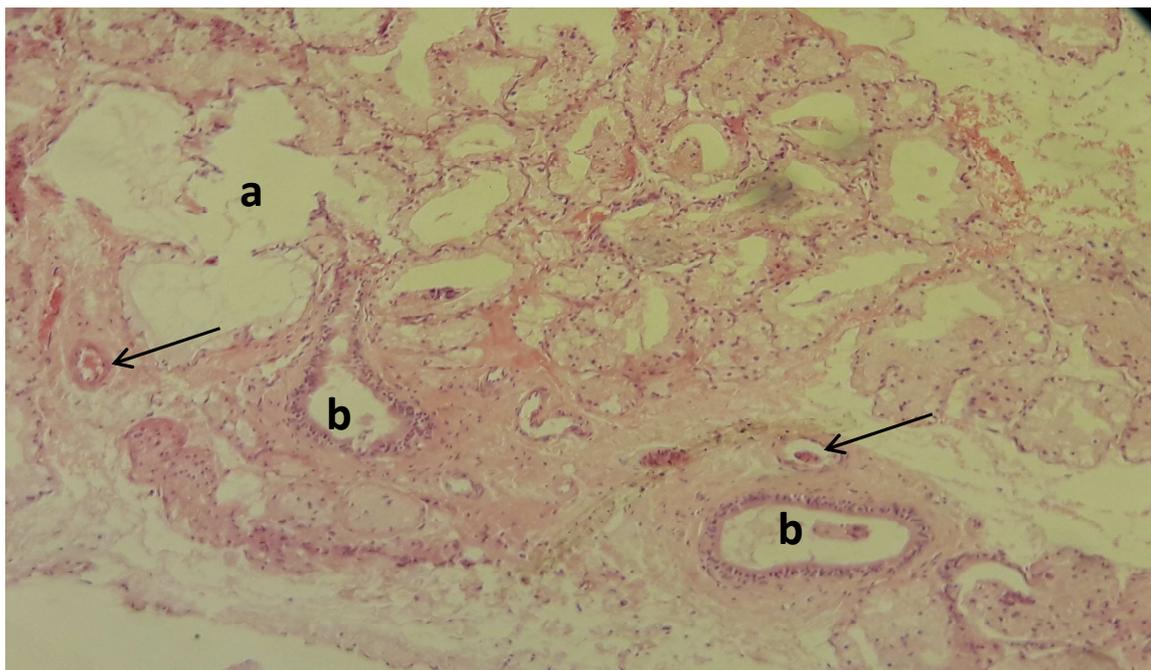


Fig. (7): Histological section of lacrimal gland in rabbit in group (II) showing some acini destroyed (a), congestion and dilation in blood vessels (arrows). Dilation in interlobular ducts (b). H & E stain (X 100).



Fig. (8): Histological section of lacrimal gland in rabbit in group (II) showing Dilation in interlobular duct (a) and blood vessels (b) and presence of cellular infiltration (arrows). H & E stain (X 400).

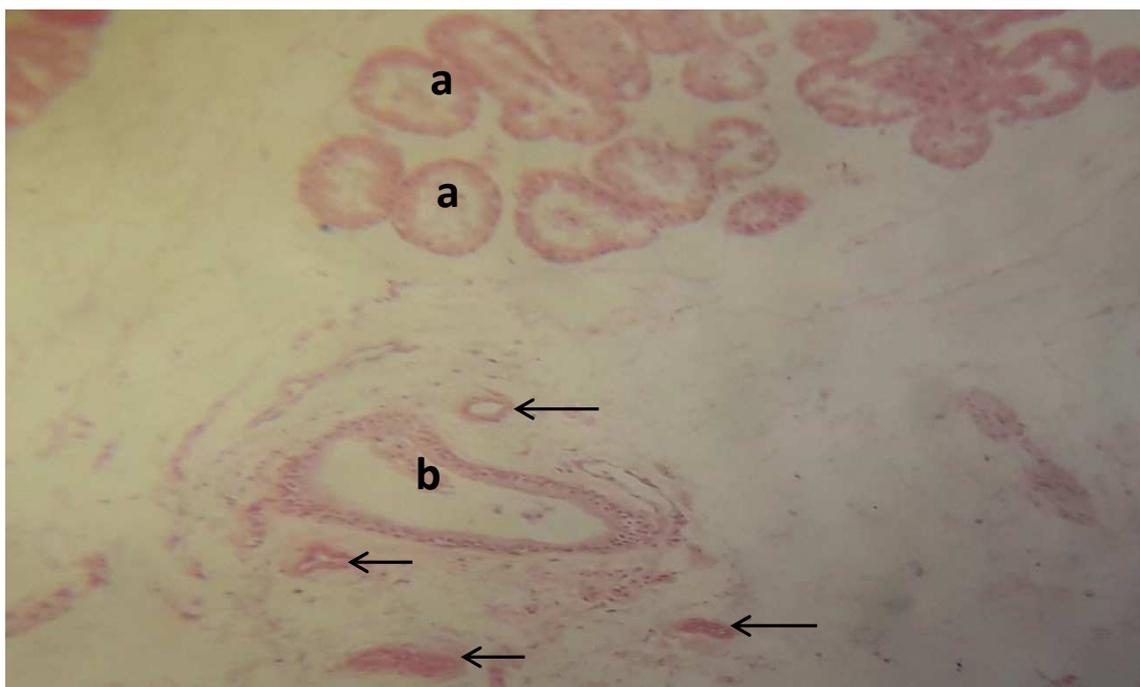


Fig. (9): Histological section of lacrimal gland in rabbit in group (III) showing acini (a), interlobular duct (b) and blood vessels (arrows). H & E stain (X 400).