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Does Oral Royal Jelly Ameliorate the Effect of Aging on the Lacrimal gland of Male Albino Rat? A Clue to the Treatment of Age-Related Dry Eye

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Abstract

In this study, the effect of aging on the structure of lacrimal gland in male albino rats and the possible therapeutic role of royal jelly were investigated. Thirty-Six male albino rats were divided into 2 groups (18 rats/group): group I (adult), group II (senile). Each is divided into 3 subgroups. Group I (A, B & C) are (adult control, Adult + distilled water& Adult+ Royal jelly) respectively. Group II (A, B&C) are (senile control, Senile+ distilled water & Senile+ Royal jelly) respectively. Rats in groups IC and IIC received given royal jelly dissolved in distilled water, by oral gavage, in a dose of 300mg/kg/day, once daily for 4 weeks. while control rats received equivalent amount of distilled water used to dissolve the royal jelly. After 4 weeks, all rats were sacrificed, lacrimal glands were dissected, the specimens were processed for paraffin & semi-thin sections and examined by light microscopy. Senile rats (group IIA&B showed apparent serous mucinous degenerative changes with loss of normal architecture. Ductal proliferation and obstructed lumina were observed. Periductal, peri acinar and peri vascular mononuclear inflammatory cell infiltrate were seen. There was dense collagen fiber deposition. Morphometric results revealed age related statistically significant decrease in the number of acini, whereas intralobular ducts showed a statistically significant increase in senile group IIA&B. Interestingly, senile rats treated with Royal jelly group IIC showed restoration of normal architecture of the gland. It was concluded that Royal jelly improved the effect of aging on lacrimal gland giving a clue to treatment of age-related dry eye.

Keywords: Senile, lacrimal gland, Royal Jelly, morphometry, semi-thin

Introduction

The lacrimal gland is an exocrine gland consists of lobules separated by loose connective tissue; each lobule is composed of multiple acini lined with columnar secretory cells and intralobular ducts that drain into approximately 8–12 excretory ducts [1] .

The lacrimal gland and ocular surface form a mucosal immune system, and both are affected by environmental factors. The quality and quantity of tear film decrease with age and dry eye is one of the most common problems in elderly patients visiting ophthalmologists [2].

Aim of the Work

The aim of this study is to investigate the histological effect of aging on the lacrimal gland of male albino rat and to observe the structural changes in the aged lacrimal gland following Royal jelly administration..

Materials and Methods

Thirty-Six male Albino rats of wistar strain were used in this study including: 18 adults with age from 3 to 6 months and weighed 180-220gm, and 18 senile aged from 18 to 24 months and weighed 350-400gm [3]. They were obtained from the Medical Research Centre of the Faculty of Medicine, Ain Shams University**.** The experiment was performed at the animal house of ***the Research Center and Bilharzial Research Unit, Faculty of Medicine, Ain Shams University***. The experimental protocol was approved by CARE (Committee of Animal Research Ethics) of faculty of medicine Ain-Shams University. Rats were housed in metallic cages 3rats/cage, exposed to normal dark/light cycle, good ventilation, suitable temperature 22°-25° c, allowed standard diet and free water access *ad libitum* under suitable environmental conditions.

Experimental design:

1. Experimental animals:

Rats were divided into two groups:
Group I (Adult group): consisted of 18 adult male rats their age from 3-6 months and were further subdivided into three subgroups:

- **Subgroup IA (Control adult):** (n=6) rats were not subjected to any procedure for 4 weeks and were used as negative control.
- **Subgroup IB (Adult+ distilled water):** (n=6) rats were given 1.5ml distilled water (vehicle) by oral gavage once daily for 4 weeks.
- **Subgroup IC (Adult+ Royal jelly):** (n=6)rats were given royal jelly dissolved in distilled water, by oral gavage, in a dose of 300mg/kg/day, once daily for 4 weeks and were used as positive control.

Group II (Senile group): consisted of 18 senile male rats aged 18-24 months and were further subdivided into three subgroups as follows:

- **Subgroup IIA (Control Senile): (**n=6) negative control senile rats that were not be subjected to any procedure for 4 weeks.
- **Subgroup IIB (Senile+ distilled water):** (n=6) control senile rats and were given 1.5ml distilled water (vehicle), by oral gavage once daily for 4 weeks.
- **Subgroup IIC (Senile+ Royal jelly):** (n=6) senile rats were given royal jelly dissolved in distilled water, by oral gavage, in a dose of 300mg/kg/day, once daily for 4 weeks.

2. Drugs:

Royal Jelly:

RJ was manufactured by (Pharco Pharmaceutical – Alexandria – Egypt) in the form of capsules 1000mg /cap. Each capsule was dissolved in 10ml distilled water and rats were given 1.5ml/ day by oral gavage which is equivalent to 300mg/kg/day of royal jelly for 4weeks [4].

Collection of specimens and tissue preparation for light microscopic study:

At the end of the experiment, all rats were sacrificed by decapitation, after being deeply anesthetized with ether according to the protocol of the Committee of Animal Research Ethics (CARE). The lacrimal gland of both eyes of each animal were carefully dissected out and immediately fixed in 10% formalin for preparation of paraffin blocks. Sections of 5 µm thick were cut and stained with Hematoxylin and Eosin (H&E) and Periodic acid-Schiff (PAS) counterstaining with hematoxylin. Mallory Trichrome stain for the lacrimal gland was done to detect the collagen fibers which stained blue, then all sections examined by light microscope**.**

Statistical studies and image analysis:

Morphometric analysis:

Five non-overlapping fields from five stained sections of five different rats were examined to identify the following:

1. Acinar count: It was measured by counting the acini in central fields under magnification 400.
2. Ductal count: It was measured by counting each type of duct in central fields under magnification 100.

Statistical analysis:

Measurements were taken using the image analyzer Leica (Q 500 MC program, Wetzlar, Germany). The mean values and standard deviations were calculated by the SPSS software version 17 (IBM Corporation, New York, USA). Analysis of variance (one-way ANOVA) was done followed by a post hoc test was used to determine which of the groups are responsible for the observed significant difference. The significance of data was determined by the P. value (probability of chance):

$P > 0.05$ was considered non-significant.
$P \leq 0.05$ was considered significant.
$P \leq 0.001$ was considered highly significant.

To exclude bias, all images were analyzed by an examiner who did not know the coding of the study groups.

Ethical Consideration:

Experimental protocols and design were carried out according to the guidelines of the Research Ethics Committee (REC), FWA 000017585, Faculty of Medicine, Ain Shams University, Egypt.

Results

In the present study, the histological findings of the lacrimal gland of adult male albino rats of subgroup IA, IB, and IC revealed that they had a similar result.

The following photos will be representative for all.

Group I (Control Adult):

Examination of Haematoxylin and Eosin-stained sections of the lacrimal gland of adult male albino rats of group I showed that the gland was covered with a thin connective tissue capsule from which short thin interlobular septa radiated into the gland substance dividing it into many lobules formed of few fibroblast in between connective tissue fibres which surrounded the acini. Each serous acinus consisted of rested on thin regular basement membrane which had basal deep stained rounded nuclei **(Fig. 1A).**

Some sections revealed serous acini together with the three components of the duct system which was consisted of the intercalated ducts that drained the acini and merged into larger intralobular ducts then became interlobular ducts which drained the lobules and embedded into a thin connective tissue septum **(Fig. 1B).**

The serous acini consisted of pyramidal-shaped cells, their nuclei appeared vesicular, euchromatic with prominent nucleoli. The acinar cytoplasm was deeply stained in the basal region and become pale granular towards the luminal side **(Fig. 1C).**

Some sections revealed that there were two types of secretory acini encountered in the lacrimal gland. Some had homogenous cytoplasm and other had vacuolated cytoplasm. The majority were serous and few of them were mucous secreting acini.

The mucous acini had highly vacuolated cytoplasm and their nuclei compressed towards the basement membrane **(Fig. 1D).** Each type of the duct system had a different epithelial lining and different luminal size. Both intercalated and intralobular ducts had the same lining by one layer of cuboidal cells with acidophilic cytoplasm and pale stained nuclei. The intercalated ducts had the smallest lumina. Both were in close proximity with the acini and surrounded with minimal connective tissues. The interlobular ducts had the largest lumina and they were lined by two to three layers of cuboidal cells with acidophilic cytoplasm and pale stained nuclei. These ducts are surrounded by more connective tissues containing thin walled blood vessels **(Figs. 1D).**

Examination of Toluidine blue stained semi - sections of the lacrimal gland of adult male albino rats of group IA showed that Some sections revealed serous acini that were consisted of pyramidal-shaped cells, their nuclei appeared vesicular, euchromatic with prominent nucleoli. The acinar cytoplasm was deeply stained in the basal region and become pale granular towards the luminal side **(Fig. 2A).** The serous acini besides the two types of the duct system had a similar epithelial lining but different luminal size. Both intercalated and intralobular ducts had the same lining by one layer of cuboidal cells with acidophilic cytoplasm and pale stained nuclei. The intercalated ducts had the smallest lumina. Both were in close proximity with the acini and surrounded with minimal connective tissues **(Fig. 2B).** The interlobular ducts had the largest lumina and they were lined by two to three layers of cuboidal cells with acidophilic cytoplasm and pale stained nuclei. These ducts are surrounded by more connective tissues containing thin-walled blood vessels and most acini are serous **(Fig. 2C).**

Group II (senile group):

Examination of H&E-stained sections of the lacrimal gland of senile male albino rats of subgroup IIA and IIB showed that they had a similar histological finding. The following photos will be representative for both.

Group II A& B (Control Senile):

Examination of H&E-stained sections of the lacrimal gland of senile male albino rats **group II A&B** revealed that the lacrimal gland was covered with a thick connective tissue capsule, from which thick interlobular septa radiated into the gland substance divided it into many widely separated lobules. The thick septa infiltrated with many fat cells.

The thick capsule had many fibroblasts encountered between connective tissue fibers. Most sections showed apparent serous degenerative changes that was frequently seen with loss of its normal architecture in H&E and semi-thin sections **(Figs.4A & 5A, 5B, 5C).** Some sections showed apparent mucinous degenerative transformation in the H&E sections only in **(Fig.4A).**

The degenerated serous acini had less granular cytoplasm with dark stained pyknotic nuclei **(Fig. 3A).** In some sections, many ducts were dilated and there was an apparent increase in their number (ductal proliferation). Fatty cells infiltrate was seen between acini with its characteristic signet ring nuclei. Also, there were a focal area of thinning of the epithelial lining of some ducts **(Fig. 3B).** Their lumina were full of homogenous structure less eosinophilic secretions obstructing them**(Figs. 3B& 3C).** There was a focal area of acinar atrophy as well as periductal, peri acinar and peri vascular mononuclear inflammatory cell infiltrate **(Fig. 3C)**

Other sections revealed mononuclear inflammatory cell infiltrate in the interlobular space, which had binucleated nuclei and eosinophilic cytoplasm. Periductal and peri vascular thickened dense connective tissue fibers (fibrosis) were observed **(Fig. 3D).**

Some sections revealed mucinous acini with multiple cytoplasmic vacuolations were seen pushing the nuclei peripherally **(Fig. 4A**). Wide interlobular space with many inflammatory cells were seen. Few spindle-shaped fibroblasts with many cytoplasmic processes, had rod shaped nuclei and eosinophilic cytoplasm were noticed. Rounded lymphocytes had rounded nuclei and thin rim of eosinophilic cytoplasm were seen together with peri-acinar thickened dense connective tissue fibers **(Fig. 4B).**

The semi -thin sections showed most of acini were serous with loss of typical appearance of pyramidal arrangement, the apical granular cytoplasm was hardly detected in most of sections with highly vacuolated cytoplasm. Slight nuclear changes as compared to that of the control were seen **(Fig. 5A).** There were dilated interlobular duct showed homogenous structure less material obstructing them. Periductal and per acinar mononuclear inflammatory cell infiltrate was observed. Many dilated congested blood vessels were Noticed **(Figs. 5B & 5C).** Some acinar cells showed small darkly stained nuclei with less apical granular cytoplasm **(Fig. 5C).**

Group II C (Senile group + Royal Jelly):

Examination of H&E-stained sections of the lacrimal gland of senile male albino rats treated with Royal jelly group IIC showed restoration of normal architecture of the gland. Sections revealed thin capsule consisted of loose less compact collagen fibres with few fibroblasts. There was restoration of normal architecture of serous acini **(Fig. 6A).** Some sections showed restoration of normal inter-acinar and interlobular spaces and secretory acini. There was apparent restoration of the duct system to the adult size and number, although, there were minimal periductal inflammatory cells infiltrate **(Fig. 6B).** Apparently normal intercalated duct and some acini showing binucleated cells. Notice few fibroblasts in thin connective tissue septa between lobules **(Fig. 6C).** Most secretory acini were closely packed and had basal deep stained nuclei. The myoepithelial cells were easily detected and surrounded both the acini and the intercalated ducts **(Fig. 6D).**

The semi -thin sections showed most of acini were serous with preservation of the normal acinar and ductal architecture **(Figs. 7A & 7B).**

Results of Mallory Trichrome stained sections:

Examination of Mallory Trichrome stained sections of the lacrimal gland of adult male albino rats of group I showed thin connective tissue capsule, few collagen fibres in the thin septa between lobules, and thin-walled blood vessels **(Fig. 8A**). The intralobular ducts surrounded by little amount of collagen fibres which became well developed around the interlobular duct. Also, there are a network of delicate collagen fibres partially separating the acini from the neighbouring acini and ducts **(Fig. 8B).**

Examination of Mallory Trichrome stained sections of lacrimal gland of senile male albino rats **group II A&B** showed dense collagen fibres in the thick capsule **(Fig. 9A),** surrounding the gland and thick septa between lobules **(Figs. 9A and 9B**). Also, many collagen fibres were seen surrounding the duct system and the thick-walled blood vessels **(Fig. 9B**).

Examination of Mallory Trichrome stained sections of senile male albino rats treated with Royal jelly **group IIC** showed apparently restoration of normal amount of collagen fibres in the lacrimal gland as in adult group. There was relatively thin connective tissue capsule formed of many collagen fibres with thin septa radiating between lobules formed of few thin collagen fibres **(Fig. 10A).** Also, there were few collagen fibres surrounds the interlobular ducts and the thin-walled blood vessel **(Fig. 10B).**

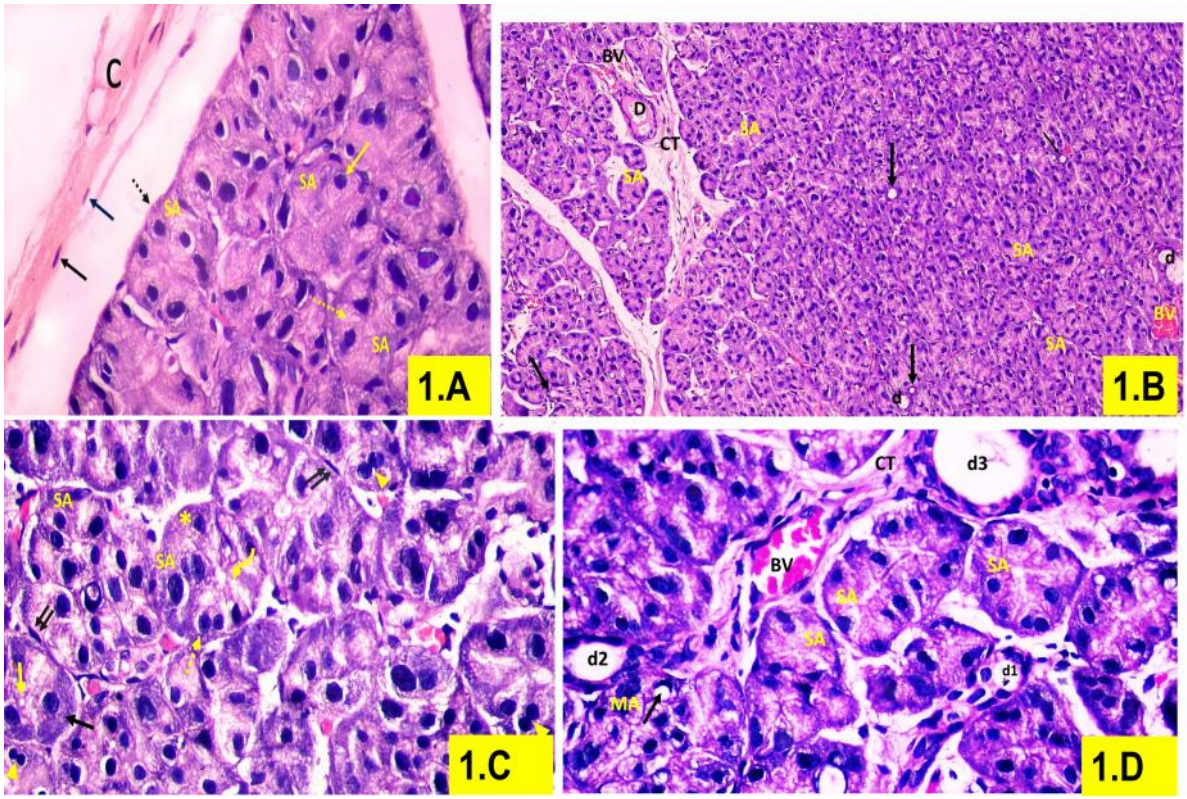


Fig. (1A): A photomicrograph of a section in the lacrimal gland of adult male albino rat of group I showing thin capsule **(C)** with few fibroblasts **(black arrow)** encountered between connective tissue fibers. Notice the serous acini **(SA)** having basal deep stained nuclei **(yellow arrow)** and homogenous granular cytoplasm **(dotted yellow arrow).** pyramidal-shaped acinar cells rested on thin regular basement membrane **(dotted black arrow).**
(Hx. &E. X**400).**
Fig. (1B): A photomicrograph of a section in the lacrimal gland of adult male albino rat of group I showing the duct system, the inter lobular duct **(D)** impeded into thin connective tissue **(CT),** intralobular **(d)** and intercalated **(arrows)** close to acini. Notice many secretory serous acini with basal deep stained nuclei and homogenous cytoplasm **(SA).** Notice also, few small blood vessels **(BV).**
(Hx. &E. X**100)**
Fig. (1C): A photomicrograph of a section in the lacrimal gland of adult male albino rat of group I showing many serous acini **(SA)** consists of pyramidal shaped cells**(*)** which have basal rounded nuclei with prominent nucleoli **(dotted arrow)** and narrow lumen **(yellow arrow).** The acinar cytoplasm is deeply stained in the basal region **(black arrow)** while pale granular towards the luminal side **(oblique arrow).** The myoepithelial cells **(double arrows)** can easily detected and surrounds the acini **(SA).**Notice some acini have binucleated cells **(head arrow).**
(Hx. &E. X**400).**
Fig. (1D): A photomicrograph of a section in the lacrimal gland of adult male albino rat group I showing the duct system with different epithelial lining, the intercalated duct lined by one layer of low cuboidal epithelium and having the smallest lumen **(d1),** the intralobular duct lined by one layer of cuboidal epithelium **(d2).** The interlobular duct **(d3)** has the largest lumen, lined by two to three layers of cuboidal cells and impeded in a thin connective tissue septum **(CT)** with thin-walled blood vessel **(BV).** Notice most acini are serous **(SA)** with few mucous acini **(MA)** having vacuolated cytoplasm with the nuclei compressed towards the basement membrane **(arrow).**
(Hx. &E. X**400).**

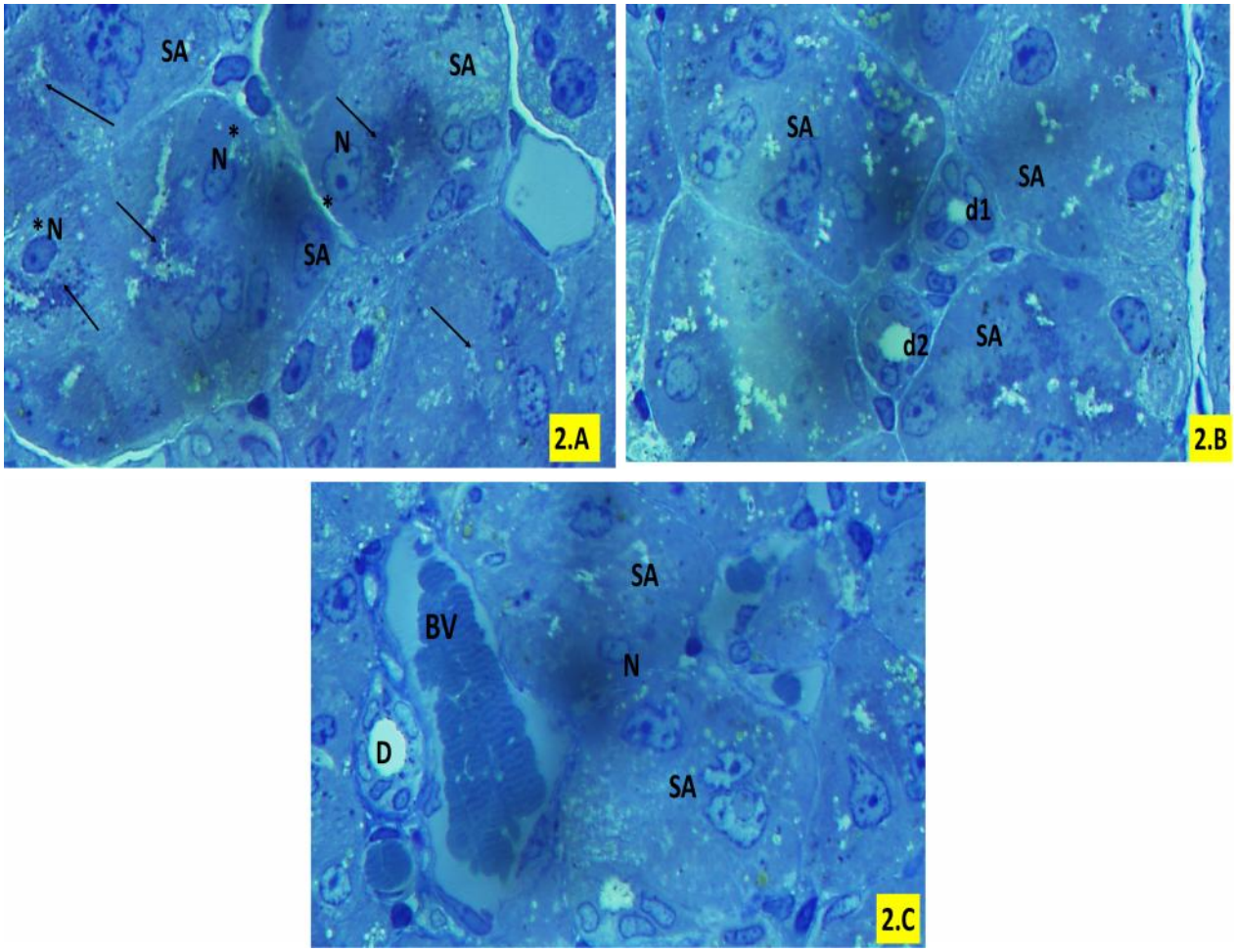


Fig. (2A): A photomicrograph of a semi-thin section in the lacrimal gland of adult male albino rat of group IA showing the serous acini **(SA)** having pyramidal acinar cells rested on thin regular basement membrane **(*)** with **vesicular**, euchromatic nucleus (N) with prominent nucleoli, basal deep stained cytoplasm and apical pale granular cytoplasm **(arrow).**
(Toluidine blueX**1000).**
Fig. (2B): A photomicrograph of a semi-thin section in the lacrimal gland of adult male albino rat of group I showing the serous acini **(SA),** Both intercalated (d1) and intralobular (d2) ducts had the same lining by one layer of cuboidal cells with pale stained cytoplasm and nuclei. The intercalated ducts (d1) had the smallest lumina. Both were in close proximity with the acini and surrounded with minimal connective tissues.
(Toluidine blueX**1000).**
Fig. (2C): A photomicrograph of a semi-thin section in the lacrimal gland of adult male albino rat of group I showing the duct system, the inter lobular duct **(D)** have the largest lumen, lined by two to three layers of cuboidal cells with acidophilic cytoplasm and pale stained nucleiwith thin walled blood vessel **(BV).** Notice most acini are serous **(SA).**
(Toluidine blueX**1000).**

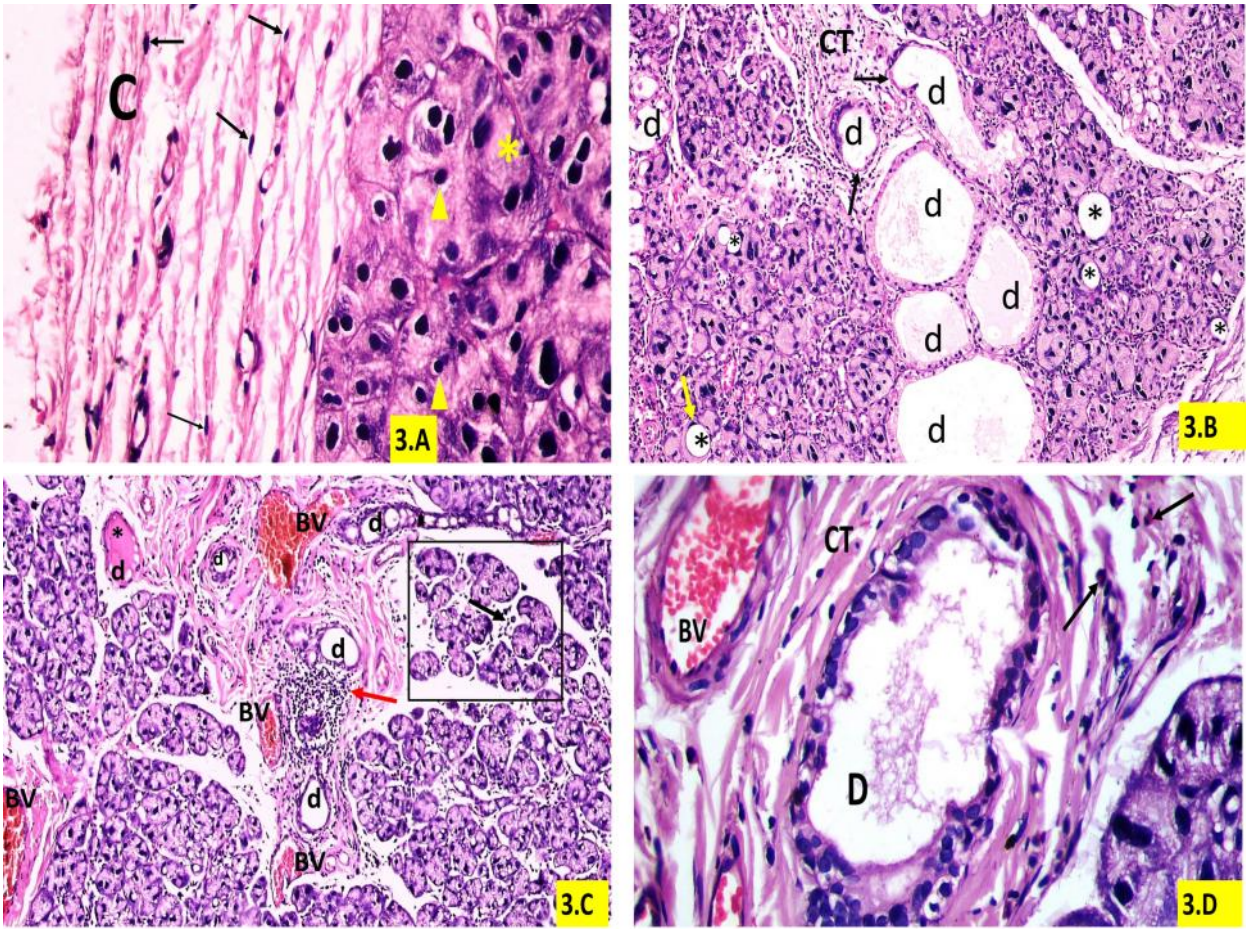
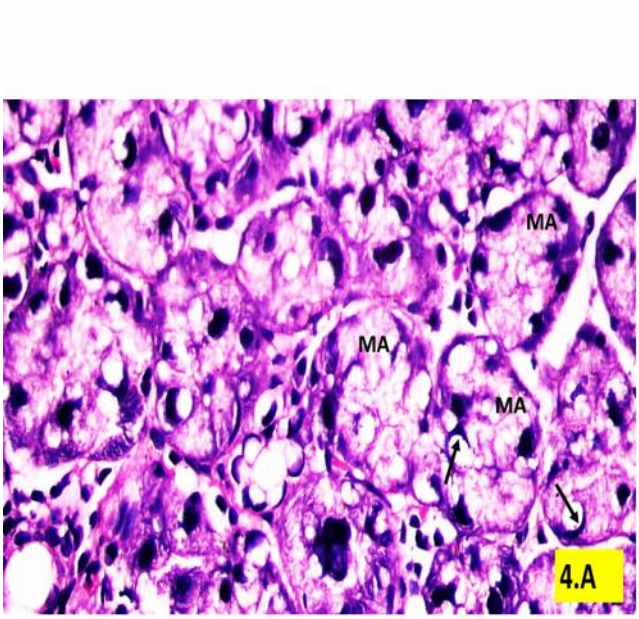


Fig. (3A):A photomicrograph of a section in the lacrimal gland of senile male albino rat group IIA&B showing thick fibrous capsule **(C)** with few fibroblasts **(arrow)** encountered between connective tissue fibers. Notice few acinar cells show small dark pyknotic nuclei **(head arrows)** with homogenous less granular cytoplasm **(*).**
(Hx. &E. X**400)**
Fig. (3B):A photomicrograph of a section in the lacrimal gland of senile male albino rat group IIA&B showing many dilated ducts with an increase in their number and their lumina are full of secretion **(d).** Few ducts show localized areas of epithelial thinning **(black arrow).** Notice many fat cells **(*)** with its characteristic signet ring nuclei **(yellow arrow).** Also, notice periductal thickened connective tissue **(CT).**
(Hx. &E. X**100)**
Fig. (3C):A photomicrograph of a section in the lacrimal gland of senile male albino rat group IIA&B showing homogenous structure less eosinophilic material **(*)** obstructing the interlobular duct **(d),** periductal **(red arrow)** and periacinar**(black arrow)** mononuclear inflammatory cell infiltrate. Notice many dilated congested blood vessels **(BV).** The inset shows focal area of loosely packed atrophic acini.
(Hx. &E. X**100)**
Fig. (3D):A photomicrograph of a section in the lacrimal gland of senile male albino rat group IIA&B showing thick-walled congested blood vessel **(BV)** and dilated interlobular duct **(D).** There are perivascular and periductal thickened dense connective tissue fibers **(CT).** Notice infiltration of neutrophils **(arrows)** in the interlobular space which have binucleated nuclei and eosinophilic cytoplasm.
(Hx. &E. X**400)**



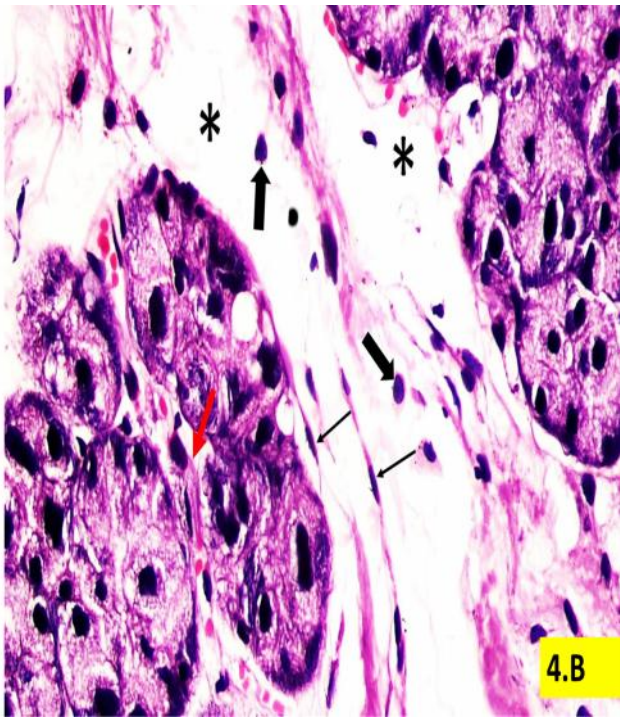


Fig. (4A): A photomicrograph of a section in the lacrimal gland of senile male albino rat group IIA&B showing that some sections present mucinous acini **(MA)** with highly vacuolated cytoplasm pushing the nuclei peripherally **(arrows).**
(Hx. &E. X**400)**

Fig. (4B):A photomicrograph of a section in the lacrimal gland of senile male albino rat group IIA&B showing wide interlobular space **(*)** with many inflammatory cells. There are few spindle-shaped fibroblasts **(thin arrow)** with many cytoplasmic processes having rod shaped nuclei and eosinophilic cytoplasm. Others are rounded lymphocytes **(thick arrow)** having rounded nuclei and thin rim of eosinophilic cytoplasm. Notice peri-acinar thickened dense connective tissue fibers **(red arrow).**
(Hx. &E. X**400)**

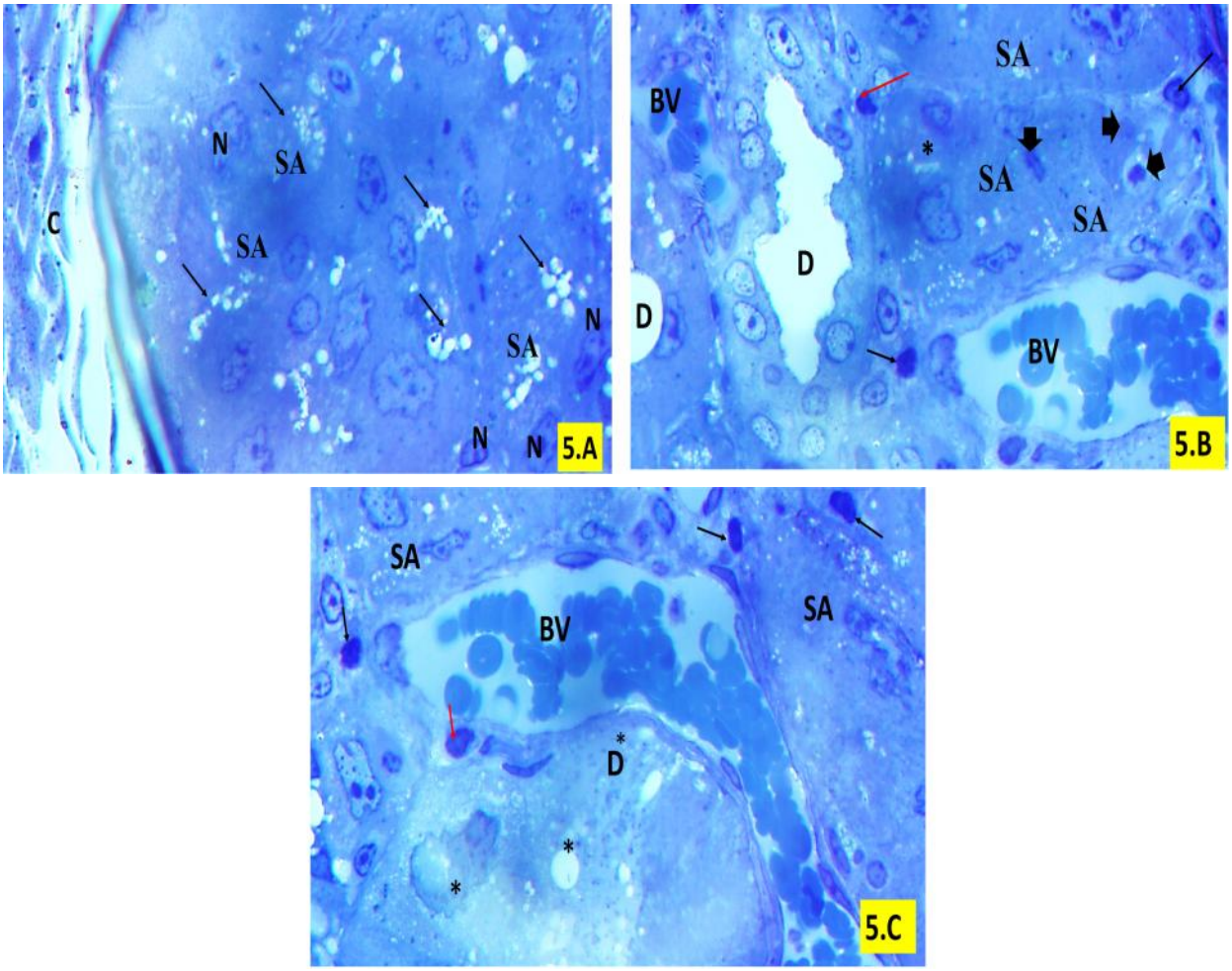


Fig. (5A): A photomicrograph of a semi-thin section in the lacrimal gland of senile male albino rat of group II A&B showing most of acini are serous (SA) with loss of typical appearance of pyramidal arrangement. The apical granular cytoplasm is hardly detected in most of sections. Highly vacuolated cytoplasm (arrows) and slight nuclear changes (N) are present. C=capsule.
(Toluidine blueX1000).

Fig. (5B): A photomicrograph of a semi thin section in the lacrimal gland of senile male albino rat of group IIA & B showing areas of degenerated serous acini (SA)with many forms of nuclear degeneration (arrowhead). Dilated interlobular duct (D) showing homogenous structure less material (*) obstructing it. periductal (red arrow) and periacinar (black arrow) mononuclear inflammatory cell infiltrate. Notice many dilated congested blood vessels (BV).
(Toluidine blueX1000).

Fig. (5C): A photomicrograph of a semi thin section in the lacrimal gland of senile male albino rat of group II A & B showing Many dilated interlobular duct (D) with an increase in their number are noted .BV=blood vessels. Note: periductal (red arrow) and periacinar (black arrow) mononuclear inflammatory cell infiltrate. Notice some cells of serous acini (SA) showing small darkly stained nuclei (head arrows) with less granular cytoplasm (*).
(Toluidine blueX1000).

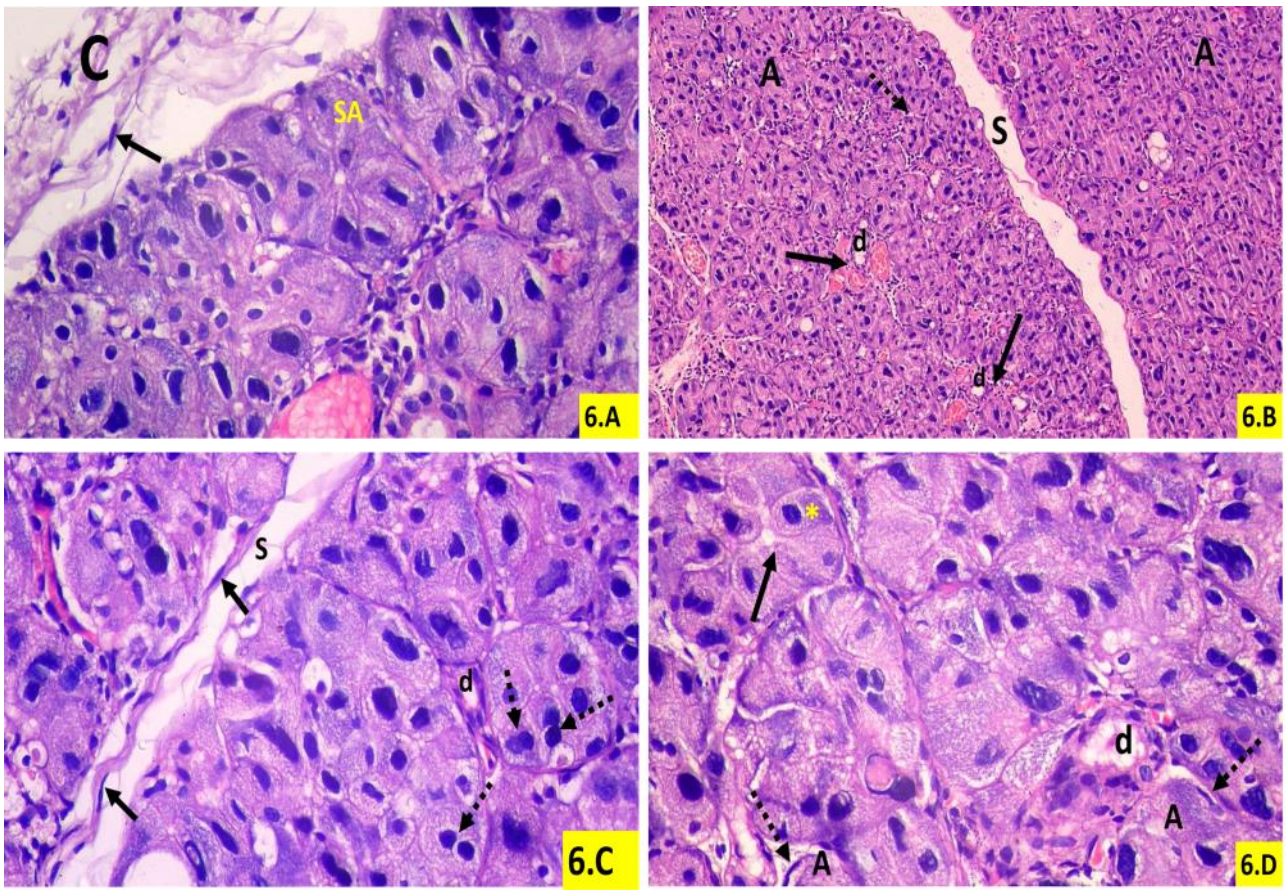


Fig. (6A): A photomicrograph of a section in the lacrimal gland of senile male albino rat group IIC treated with Royal jelly showing relatively thin capsule consists of loose less compact collagen fibers **(C)** with few fibroblasts**(arrow).** Notice restoration of normal architecture of serous acini **(SA.)**
(Hx. &E. X**400)**

Fig. (6B):A photomicrograph of a section in the lacrimal gland of senile male albino rat group IIC treated with Royal jelly showing Apparent restoration of the normal architecture. The inter-acinar space **(dotted arrow),** interlobular space **(S)** and secretory acini **(A).** There is apparent restoration of the duct system to the adult size and number **(d).** Notice minimal periductal inflammatory cell infiltrate **(arrows).**
(Hx. &E. X**100)**

Fig. (6C): A photomicrograph of a section in the lacrimal gland of senile male albino rat group IIC treated with Royal jelly showing apparently normal intercalated duct **(d)** and some acini showing binucleated cells **(dotted arrows)**. Notice few fibroblasts **(arrow)** in thin connective tissue septa **(S)** between lobules.
(Hx. &E. X**400)**

Fig. (6D): A photomicrograph of a section in the lacrimal gland of senile male albino rat group IIC treated with Royal jelly showingrestoration of normal architecture of acini. The majority are serous acini; their cytoplasm was deeply stained in the basal region **(*)** while granular pale towards the luminal side **(arrow)**. The myoepithelial cells are easily detected **(dotted arrows)** and surrounds the acini **(A).** Notice the intralobular duct **(d)** is apparently like adult size and epithelial lining.
(Hx. &E. X**400)**

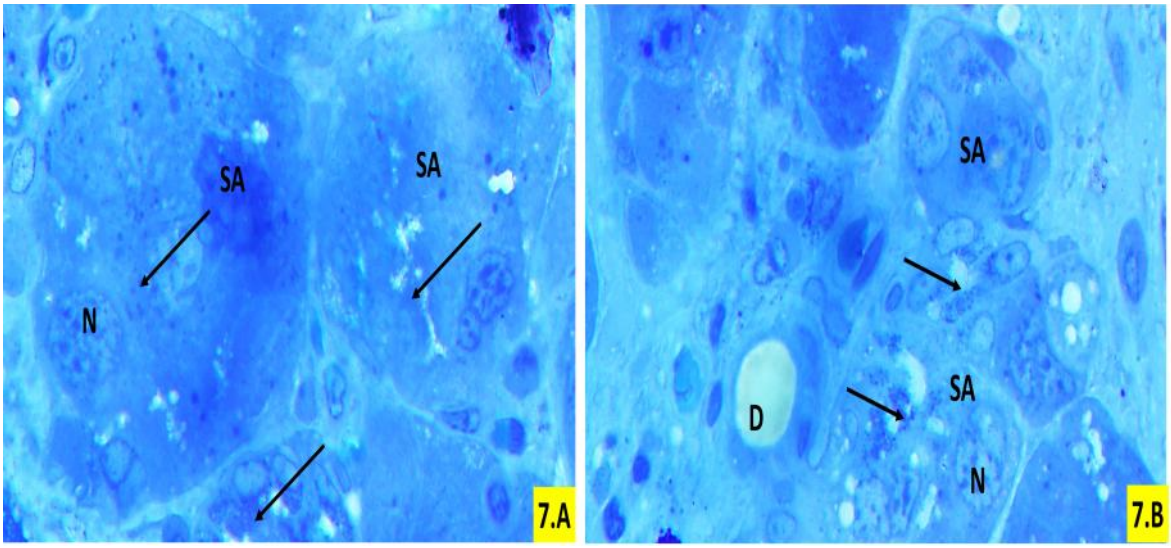


Fig. (7A): A photomicrograph of a semi-thin section in the lacrimal gland of senile male albino rat of group IIC showing apparently normal serous acini (SA). (N) =nuclei (arrows) =Apical granular cytoplasm.
(Toluidine blueX1000).
Fig. (7B): A photomicrograph of a semi-thin section in the lacrimal gland of senile male albino rat of group IIC showing apparently normal sized interlobular duct (D). (SA)= serous acini (N) =nuclei (arrows) =Apical granular cytoplasm.
(Toluidine blueX1000).

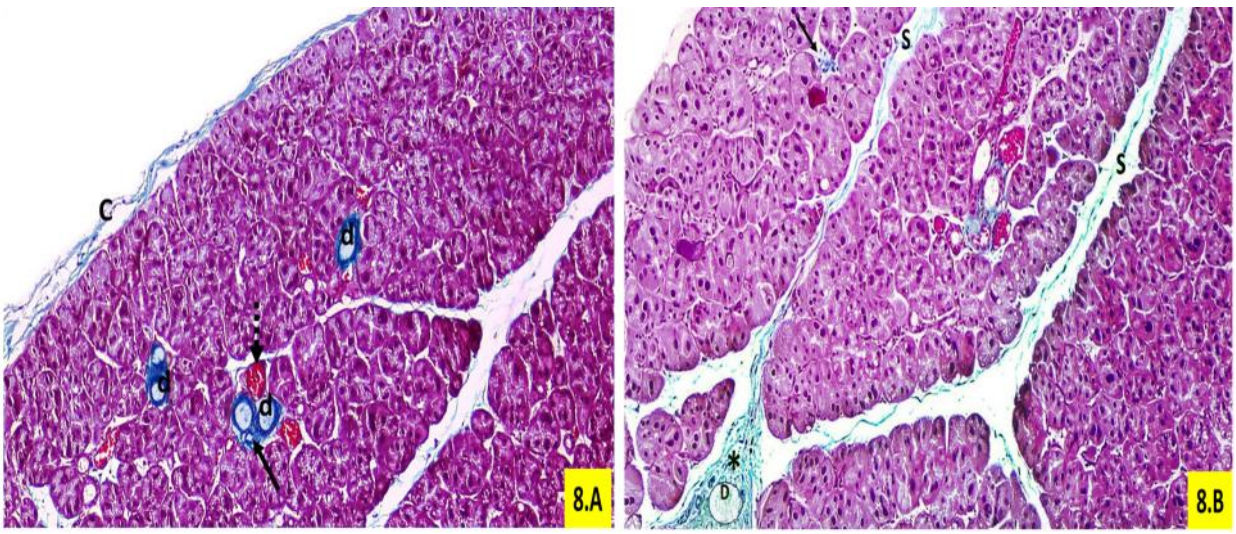


Fig. (8A): A photomicrograph of a section in the lacrimal gland of adult male albino rat of group I showing little amount of collagen fibers in the thin capsule **(C).** The intralobular ducts **(d)** are surrounded by few collagen fibers **(arrow)**. Notice thin-walled blood vessel **(dotted arrow).**
(Mallory Trichrome X**100).**

Fig. (8B): A photomicrograph of a section in the lacrimal gland of adult male albino rat of group I showing scanty collagen fibers in the thin septa **(S)** between lobules**.** There is well developed collagen fibers **(*)** around the interlobular duct **(D)**. Notice a network of delicate collagen fibers partially separating the acini from the neighboring acini and ducts **(arrow).**
(Mallory TrichromeX**100).**

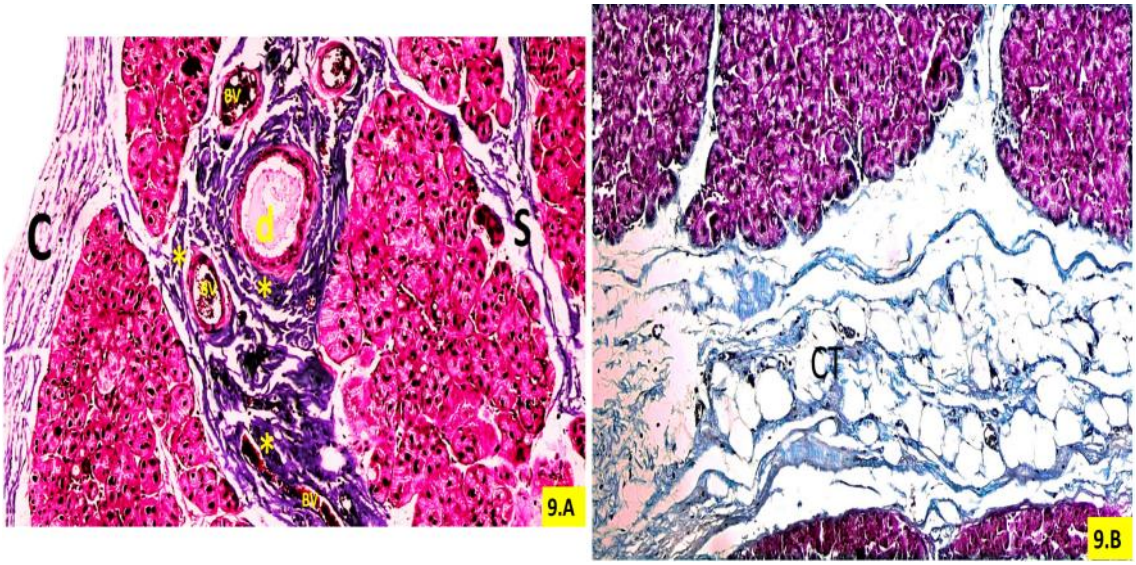


Fig. (9A): A photomicrograph of a section in the lacrimal gland of senile male albino rat group IIA &B showing dense collagen fibers in thick connective tissue capsule **(C)** and septa **(S).** Large amount of collagen fibers **(*)** surrounds the interlobar duct **(d)** and the thick-walled blood vessel **(BV).**
(Mallory TrichromeX**100).**
Fig. (9B):A photomicrograph of a section in the lacrimal gland of senile male albino rat group IIA&B showing dense collagen fibers in connective tissue septa between lobules **(CT).**
(Mallory TrichromeX**100)**

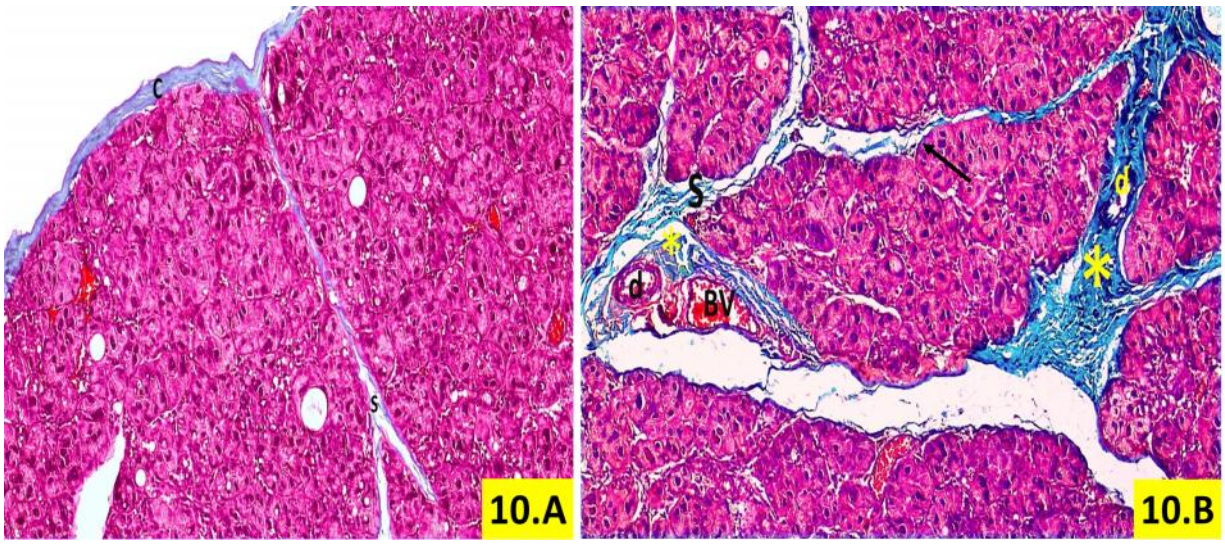


Fig. (10A): A photomicrograph of a section in the lacrimal gland of senile male albino rat group IIC treated with Royal jelly showingrelatively thin connective tissue capsule **(C)** consists of many collagen fibers. Notice few collagen fibers in thin septa **(S)** between lobules.
(Mallory TrichromeX**100)**
Fig. (10B): A photomicrograph of a section in the lacrimal gland of senile male albino rat group IIC treated with Royal jelly showing scanty collagen fibers in the thin septa **(S)** between lobules**.** There are thin collagen fibers **(*)** around the interlobular duct **(d)**. Notice a network of delicate collagen fibers partially separating the acini from the neighboring acini and ducts. blood vessels **(BV).**
(Mallory TrichromeX**100)**

Results of Periodic Acid-Schiff-stained sections Examination of Periodic Acid-Schiff-stained sections of the lacrimal gland of adult male albino rats of **group I** revealed few positive PAS reactions in form of deeply stained pink homogenous, irregular patches of secretory vesicles in the cytoplasm of acinar cells. Moreover, the duct system showed positive PAS reaction (**Fig. 11A**). Examination of Periodic acid-Schiff-stained sections of lacrimal gland of senile male albino rats **group II A& B** demonstrated dilated interlobular ducts with many positive PAS reactions in the cytoplasm of acinar cells and ducts **(Fig. 11B**).

Examination of Periodic Acid-Schiff-stained sections of senile male albino rats treated with Royal jelly group IIC revealed few secretory mucin vesicles in the cytoplasm of acinar cells that showed positive PAS reaction. Also, the interlobular duct showed positive PAS reaction **(Fig. 11C).**

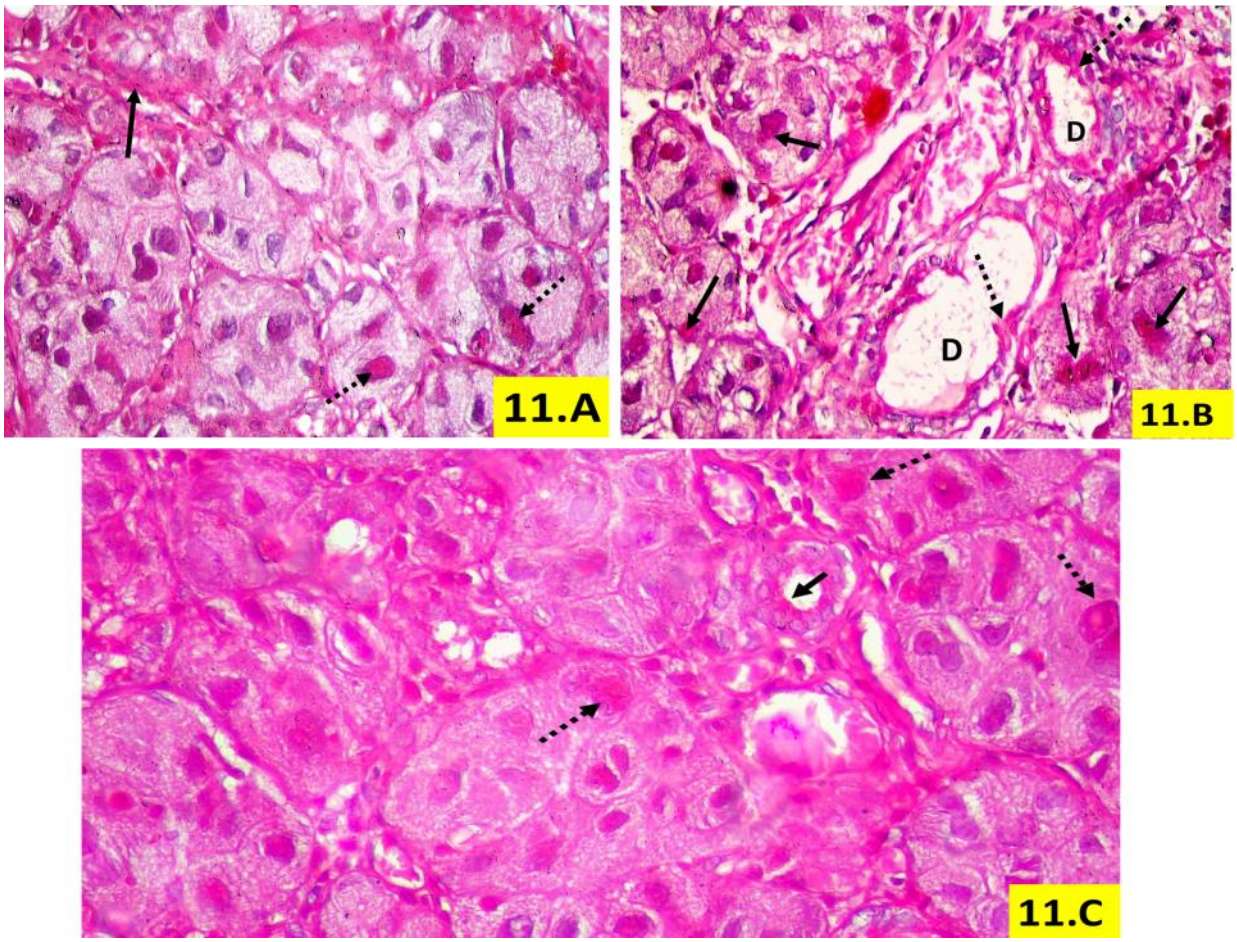


Fig. (11A): A photomicrograph of a section in the lacrimal gland of adult male albino rat of group I showing few positive PAS reactions in the form of deeply stained pink, homogenous and irregular patches of mucin vesicles **(dotted arrows)** in the cytoplasm of acinar cells. Moreover, the duct system showed positive PAS reaction **(arrow).**
(PAS counterstaining with hematoxylin X**400)**
Fig. (11B): A photomicrograph of a section in the lacrimal gland of senile male albino rat group IIA showing dilated interlobular ducts **(D)** with many positive PAS reactions in the cytoplasm of acinar cells **(arrows)** and ducts **(dotted arrows).**
(PAS counterstaining with hematoxylin X**400).**
Fig. (11C): A photomicrograph of a section in the lacrimal gland of senile male albino rat group IIC treated with Royal jelly showing few positive PAS reactions in the form of deeply stained pink, homogenous and irregular patches of mucin vesicles **(dotted arrows)** in the cytoplasm of acinar cells. Moreover, the duct system showed positive PAS reaction **(arrow).**
(PAS counterstaining with hematoxylin X**400)**

Morphometric result of the lacrimal gland

Morphometric Study in Hx & E-stained sections:

1. Acinar count:

The number of acini in Hx&E-stained sections under high power field of light microscope (magnification 400) were counted and age-related changes in the acinar number were recorded in the lacrimal gland of male albino rat. There was a statistically significant decrease (p=0.002) in the number of acini in senile group as compared to adult group. While the acini of senile rats treated with Royal jelly revealed a statistically non-significant increase (p=0.06) in their number as compared to senile group. The mean acinar count is demonstrated in **(Table1)** and **(Fig. 12).**

Table 1: Mean acinar count (number of acini) of the lacrimal gland of male albino rat in six studied groups/ high power field:

	Adult (Group IA)	Adult + distilled water (Group IB)	Adult + Royal jelly (Group IC)	Senile (Group IIA)	Senile + distilled water (Group IIB)	Senile + Royal jelly (Group IIC)
Acinar count (mean ± SD)	22 ± 3.55	22.25 ± 3.045	22.5 ± 3	11.5 ± 2.082 (p=0.002) [a]	11.25 ± 2.872	15.75 ± 3.096 (p=0.06) [b]

a) Significant decrease as compared to adult group.
Values are expressed as (mean ± SD)
P-value > 0.05 → non-significant.
P-value ≤ 0.05 → significant.
b) Non-significant increase as compared to senile group

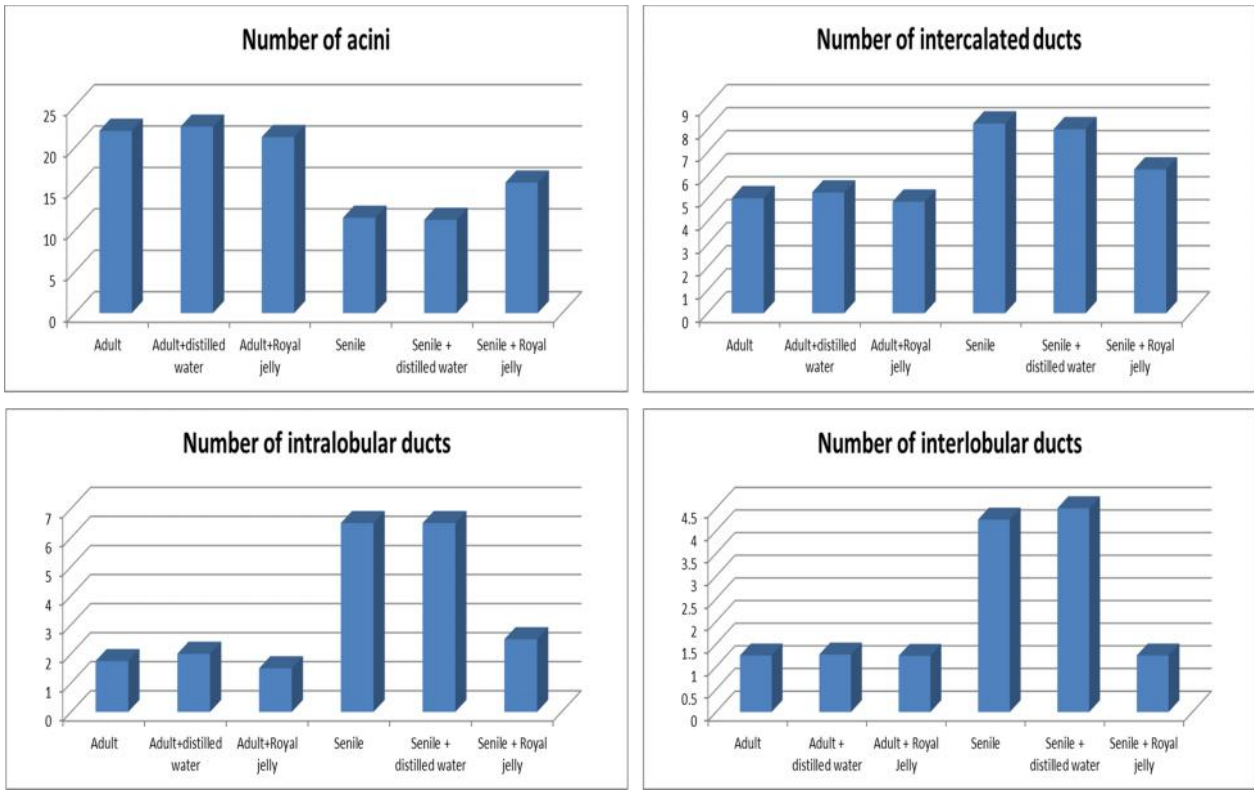


Fig. (12): Mean number of acini and ducts.

2. Ductal count:

The number of each type of the duct system of the lacrimal gland was recorded in Hx&E-stained sections under magnification 100 of light microscope. The intercalated and interlobular ducts in senile group showed a statistically non-significant increase (p=0.385) and (p=0.166) in their number as compared to adult group. However, the number of intralobular ducts showed a statistically significant increase (p=0.023) in senile group as compared to adult group. On the other hand, senile group treated with Royal jelly showed a statistically non-significant decrease (p=0.576) and (p=0.174) in the number of intercalated and interlobular ducts as compared to senile group. While the intralobular ducts showed a statistically significant decrease (p=0.044) in their number as compared to senile group. The mean duct count is demonstrated in **(Table 2) and (Fig. 12).**

Table 2: Mean ductal count of the lacrimal gland of male albino rat in six studied groups/ low power field:

Interlobular duct (mean ± SD)	Intralobular duct (mean ± SD)	Intercalated duct (mean ± SD)	Duct count
1.25 ± 1.258	1.75 ± 1.26	5 ± 1.83	Adult (Group IA)
1.05 ± 1.44	1.55 ± 1.33	5.32 ± 1.39	Adult + distilled water (Group IB)
1.23 ± 1.15	1.46 ± 1.2	5.25 ± 1	Adult + Royal jelly (Group IC)
4.25 ± 3.593 (p=0.166) [c]	6.5 ± 2.891 (p=0.023) [b]	8.25 ± 6.702 (p=0.385) [a]	Senile (Group IIA)
4.5 ± 2.38	6.5 ± 2.38	8 ± 7.118	Senile + distilled water (Group IIB)
1.25 ± 1.5 (p=0.174) [f]	2.5 ± 1.291 (p=0.044) [e]	6.25 ± 0.957 (p=0.576) [d]	Senile + Royal jelly (Group IIC)

Values are expressed as (mean ± SD)
P-value > 0.05 → non-significant.
P-value ≤ 0.05 → significant.

a) Non-significant increase as compared to adult group.
b) Significant increase as compared to adult group.
c) Non-significant increase as compared to adult group.
d) Non-significant decrease as compared to senile group.
e) Significant decrease as compared to senile group.
f) Non-significant decrease as compared to senile group.

Discussion

The present study revealed that aging had a remarkable effect on the structure of the lacrimal gland. A statistical increase in the number of different parts of the duct system were recorded which was significant in the intralobular ducts and non-significant in the intercalated and interlobular ducts. Also, the number of secretory acini showed a statistically significant decrease in their number in senile group as compared to adult group.

This work demonstrated that the lacrimal gland of senile rat was covered with thick connective tissue capsule from which thick interlobular septa radiated into the gland substance divided it into many widely separated lobules, this was consistent with **Draper et al. [5],** who stated that there was evidence of progressive morphological changes when the rat aged. These changes included thickening of the connective tissue sheath.

In this study, some sections of lacrimal gland of senile rat revealed that there was peri acinar, periductal, and peri vascular mononuclear inflammatory cell infiltrate in form of lymphocytes. This agreed with **Obata [2],** who stated thatfocal lymphocytic infiltration in the human lacrimal gland suggests subclinical dacryoadenitis and he added that periductal lymphocytic infiltration is the earliest histopathological finding in dacryoadenitis. These mononuclear inflammation cell infiltrate and fibrosis are the most prominent feature of the existing chronic inflammation.

In the present study senile lacrimal gland showed many dilated ducts with ductal proliferation. Also, atrophic ductal epithelium was found in some ducts whose lumina were full of secretions obstructing them. Peri-acinar, periductal, and peri vascular fibrosis were recorded in some sections. This was explained with **Obata [2],** who stated that periductal fibrosis in human lacrimal gland may be an important factor related to the decrease in outflow of tear fluids. Atrophic ductal epithelium is often associated with periductal fibrosis. These ductal pathological changes may interfere with electrolyte and water secretion as ductal epithelial cells are considered responsible for this function.

In addition, **Draper et al. [5],** found that patchy destruction of ductal and vascular tissues in rat lacrimal gland occurs with aging. Also, enlargement of lacrimal ducts can occur.

Moreover, **Rocha et al. [6],** stated that aging of human and rat lacrimal gland are accompanied by dilation and increased tortuosity of secretory ducts which lead to ductal obstruction. These ductal and acinar changes occur in the same region, suggesting that the ductal obstruction could account for the acinar atrophy. Loss of efferent nerves or nerve function play a role in the sustained loss of secretion at older ages.

The present study showed that the parenchyma of senile lacrimal gland was composed of many widely separated acini with focal areas of acinar atrophy. This was consistent with, **Nebbioso et al. [7],** who reported that aging, leads to a decrease in the acinar diameter and altering the constitution of meibum secretion. Moreover, with aging, atrophic involution of the glandular unit was observed and then a progressive dysfunction of the secretory activity occurs.

Cavallotti and Cerulli[8], reported that both inflammation and neural dysfunction may play crucial roles in age-related human lacrimal gland dysfunction. Alteration of both sensory nerves innervating the cornea and autonomic nerves innervating the lacrimal gland could cause decreases in tear secretion.

Another finding was recorded in this study in senile lacrimal gland was a degeneration of the secretory acini which was frequently seen with poor content of secretory granules. Most of acini were mucinous with multiple cytoplasmic vacuolations were seen pushing the nuclei peripherally. While the remaining, few serous acini had ill-defined cell boundaries with several degrees of nuclear degeneration and less granular homogenous cytoplasm.

All these findings are explained by **Bakeeva et al. [9],** who stated that during aging, the synthesis and secretion of proteins decrease in rat lacrimal gland. The acinar cells start to produce and secrete a mucous product which causes aberrations in the tear film of the eye. Aging of the rat lacrimal gland is accompanied by secretory granular changes due to morphological changes in the rough endoplasmic reticulum (RER) and Golgi apparatus of acinar cells.

In addition, **Draper et al. [5],** said that in adult LG of rat the majority of acini were serous with a few seromucous acini and even fewer mucous acini. In aged LG there were significant reductions in serous acini and there are marked increases in the percentage of occurrence of mucous acini. There is not only structural damage and chronic inflammation to the rat lacrimal gland with aging, but also possible re-differentiation of acini from serous to seromucous and then to mucous acini. Furthermore, there is a reduction or inability of the acini to synthesize and to secrete protein from glands of aged rats compared to glands of adult rats.

Draper et al.[5], reported thatthe decrease in protein secretory granules in the lacrimal acini of senile rats was associated with changes in the organization of the RER. It might lead to a reduction or an inability of the acini to synthesize protein in tears of senile rats. These changes may help to explain the phenomenon of reduced protein in tears secretion with aging which is a part of the etiology of dry eye syndrome.

The impact of dry eye on the lifestyle of aged peoples was clarified by **McClellan et al. [10],** who stated that visual impairments in the elderly have been associated with limitations in physical abilities, falls, hip fractures, and death.

In this work RJ was used as a honeybee product in treating senile lacrimal gland instead of pharmaceutical treatment. RJ was given by oral route for better systemic absorption and distribution to all component of the lacrimal gland. Apparent restoration of normal architecture of the gland was recorded, including restoration of the serous acini and the duct system to the adult size and number.

A statistically non-significant increase in the number of secretory acini was found. A statistically decrease in the number of different parts of the duct system were recorded which was significant in the intralobular ducts and non-significant in intercalated and interlobular ducts in RJ treated group as compared to senile group.

This was explained by **Imada et al. [11],** whostated that RJ increases the protein secretion from rat lacrimal acinar cells, and he added that RJ maintained the acinar cell density as the same level as the normal value.

In the present study, senile group treated with RJ showed that the majority of acini were serous which had basal nuclei and granular cytoplasm.

Some acini showing binucleated cells denoting actively regenerating cells, this was consistent with **Inoue et al. [4],** who stated that RJ increases the secretory vesicles filled the acinar cells of the rat LG and he added that oral RJ supplementation for 8 weeks can improve tear secretion, as assessed by schirmer score, in patients with dry eye symptoms.

In the present work, few inflammatory cells between acini still found may be due to the short period of this study. According to, **Kohno et al. [12],** the improvement of microstructure of LG in senile group treated with RJ referred to the anti-inflammatory actions of RJ through inhibiting proinflammatory cytokine production by activated macrophages.

The present study showed one section with dilated interlobular duct with exfoliated ductal epithelial cells in its lumen. Exfoliation of epithelial cells means regeneration of the ductal epithelium **[13]** and more improvement will be suspected with the increase in the duration of treatment.

The dilatation in interlobular duct in RJ treated group was explained by **Van Haeringen[14],** who reported that it may be caused by stenosis of the excretory duct in the fornix of the conjunctiva.

In addition, **Pasupuleti et al. [15],** RJ has also been reported to exhibit a novel function of tear secretion from LGs due to anti-inflammatory or anti-oxidative effects. RJ is a promising fundamental alternative for increasing tear secretion and protecting LG function.

More recently, **Kocot et al. [16],** reported that the mechanism of its antioxidant activity of RJ is due to very important ingredients which are flavonoids and phenolic compounds. It is a scavenger of free radicals due to its linoleic acid oxidation, and superoxide dismutase activity.

Author contributions:

All authors have contributed in conceptualization, data acquisition, data analysis, drafting of the manuscript, and Approval of the final version of the manuscript.

Conflict of interest:

No potential conflict of interest relevant to this article was reported.

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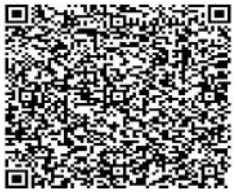	Website: www.ijcrims.com
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