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ORIGINAL ARTICLE

L-carnosine mitigates interleukin-1α-induced dry eye disease in rabbits via its antioxidant, anti-inflammatory, antiapoptotic, and antifibrotic effects

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ABSTRACT

Objective: To elucidate the implications of L-carnosine on interleukin-1α (IL-1α)-induced inflammation of lacrimal glands (LGs).

Materials and methods: Forty rabbits were divided equally into four groups: control group (G1), IL-1α (G2), L-carnosine (G3), and L-carnosine plus IL-1α (G4). Several clinical, histopathological, immunohistochemical, morphometric, and biochemical investigations were performed, followed by statistical analysis to diagnose the presence of dry eye disease (DED).

Results: The LGs of G2 rabbits showed degeneration of the acinar cells, increased deposition of collagen fibers, and marked immunoexpression of FasL; elevated levels of interferon-γ, tumor necrosis factor-α, transforming growth factor-β1, and malondialdehyde; and decreased levels of glutathione peroxidase, superoxide dismutase, catalase, and reactive oxygen species compared with those of G1 rabbits. In contrast, administration of L-carnosine to G4 rabbits revealed marked improvement of all previously harmful changes in G2 rabbits, indicating the cytoprotective effects of L-carnosine against IL-1α-induced inflammation of LGs.

Conclusions: IL-1α induced inflammation of LGs and eye dryness via oxidative stress, proinflammatory, apoptotic, and profibrotic effects, whereas L-carnosine mitigated DED through antioxidant, anti-inflammatory, antiapoptotic, and antifibrotic effects on LGs. Therefore, this work demonstrates for the first time that L-carnosine may be used as adjuvant therapy for the preservation of visual integrity in patients with DED.

HIGHLIGHTS

- IL-1α induced dry eye disease through its oxidative stress, proinflammatory, apoptotic and profibrotic effects on the lacrimal glands of rabbit.
- L-carnosine has antioxidant, anti-inflammatory, antiapoptotic and antifibrotic effects.
- L-carnosine mitigated IL-1α induced dry eye disease via elevating the levels of FasL, IFN-γ, TNF-α, TGFβ1 and MDA as well as reducing the levels of antioxidants (GPx, SOD, and catalase) and ROS in the lacrimal glands of rabbit.
- L-carnosine could be used as a novel adjuvant therapy for the treatment of dry eye disease.

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1. Introduction

Dry eye disease (DED) is a common, multifactorial, chronic, inflammatory visual problem that affects millions of people, causing deleterious effects on the patient's vision and quality of life[1,2]. It is characterized by metabolic and immune dysregulation of the lacrimal glands (LGs), Meibomian glands, and conjunctiva[3]. LGs consist of tubular acini of acinar, ductal, and plasma cells, which secrete numerous proteins into the tear film[4]. Although the mechanisms of insufficient secretion from the LGs and DED are poorly understood, chronic inflammation of the LGs is a major contributor to corneal surface damage[5]. Therefore, inhibition of inflammation in the LGs plays a crucial role in providing a healthy microenvironment to the cornea[6,7].

In this regard, DED is subdivided into evaporative and aqueous tear-deficient subtypes[8,9]. The evaporative subtype occurs because of the poor quality of the tear lipid layer, inducing rapid evaporation of the tear film from the ocular surface[10,11]. In contrast, the aqueous tear-deficient dry eye disease (ADDED) subtype results from impaired LG secretion with inadequate lubrication of the corneal surface, ocular inflammation, corneal ulceration, and loss of vision[12].

Remarkably, antigen-presenting dendritic cells produce interleukin-12, which modulates T-helper 1 cells to enhance the expression of interferon-γ (IFN-γ) and interleukin-1α (IL-1α), increased in the LG tissues in response to invasion by different microbes and neoplastic cells[13,14]. IL-1α is mainly produced by epithelial cells and activated macrophages, which induce inflammation and play a central role in

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regulating the immune response by activating tumor necrosis factor-alpha (TNF-α)[15]. In addition, IFN-γ is a specific diagnostic biomarker for DED, as IFN-γ levels are increased in the tears of inflamed LGs and activated B cells (a major immune mediator of DED)[16,17]. Moreover, transforming growth factor-β1 (TGF-β1) modulates fibrogenic properties to promote tissue repair and fibrosis[18]. Besides the effects of IL-1α and IFN-γ, oxidative stress is another leading mechanism of chronic inflammation that damages goblet cells and leads to tear-film instability[19]. Patients who complain of chronic LG inflammation suffer from signs of ADDED due to a reduction in tear secretion[20].

Despite the high prevalence of DED and its association with the eyelids, Meibomian gland, and conjunctival problems, there is no gold standard therapeutic regimen. Additionally, the DED treatment protocol requires proper hygiene of the eyelids, oily eye drops, and topical antibiotics. Thus, natural antioxidants, such as L-carnosine (a natural endogenous biological antioxidant present in the muscles, kidneys, and brain), may be a novel alternative to ameliorate the hazardous side effects of DED[21–25]. Indeed, the antioxidant activity of L-carnosine prevents oxidative tissue damage, and its anti-inflammatory activity protects tissues from numerous inflammatory injuries[26–28]. Hence, this study elucidated the role of L-carnosine against IL-1α-induced inflammation of LGs in adult male rabbits through various clinical, histopathological (HP), immunohistochemical (IHC), morphometric, and biochemical investigations.

2. Materials and methods

2.1. Animals

The current study was performed on adult (3 months old) male New Zealand white rabbits for 30 days. Rabbits were housed in metal cages at 21 °C in the animal house of the College of Applied Medical Sciences (CAMS), Qassim University (QU), Saudi Arabia. The rabbits' weight was 1.5–2 kg; they were fed a routine chow diet and tap water *ad libitum* and housed under a 12 h light/dark cycle. The study was approved by the Research Ethics Committee of QU (No. 1411-CAMS-2016–1-12–1) and performed in accordance with the guidelines of the National Institutes of Health (NIH, NIH Publication No. 8023) for the care and use of laboratory animals[29].

2.2. Drugs and materials

L-carnosine (500 mg/capsule) was purchased from Nova Nutritions (Egham, UK), whereas the standard Schirmer's tear test (STT) strips (200 strips with 35 mm gradations) were purchased from Schering-Plough (Kenilworth, NJ, USA). Anti-rabbit IFN-γ (E-EL-RB0679), TNF-α (E-EL-RB0011), and TGF-β1 (E-EL-0162) were purchased from Elabscience (Houston, TX, USA), and recombinant IL-1α protein (ab119165), polyclonal anti-Fas ligand primary antibody (ab15285), biotinylated goat secondary antibody (ab64256), malondialdehyde (MDA, ab233471), superoxide dismutase (SOD, ab65354), glutathione peroxidase (GPx, ab102530), and catalase activity (ab83464) enzyme-linked immunosorbent assay kits were purchased from Abcam (Cambridge, UK).

2.3. Experimental design

Forty rabbits were divided into four equal groups ($n = 10$). Rabbits in the control group (G1) received 2 mL distilled water (DW)/kg/day orally, while those of group two (G2) were injected in the exorbital LGs with 1 μg IL-1α/week[30]. In contrast, rabbits in group three (G3) received 10 mg L-carnosine orally (dissolved in 2 mL DW/kg/day), while those in group four (G4) were treated with the same doses of IL-1α and L-carnosine, as in G2 and G3[31]. At the end of the experiment, all rabbits were sacrificed under general anesthesia (intraperitoneal injection of 35 mg pentobarbital sodium/kg/body weight), and the excised LGs were processed for HP, IHC, and biochemical investigations.

2.4. Clinical measurement of tear volume using STT strips

The tear volume was evaluated at the beginning and end of the experiment by examining the eye using the standard STT, which indicated the efficacy of tear production of the LGs. STT strips were inserted into the lower temporal conjunctival sac of each eye after pulling down the lower eyelid for 2 min. The length of tear wetting on the STT (mm/2 min) was measured and recorded to determine the rate of tear production[32].

2.5. Evaluating the effects of L-carnosine on DED features in the LGs by HP analysis

A specimen from the LGs of each rabbit was processed to obtain 4-μm paraffin sections, which were deparaffinized, rehydrated, and stained with hematoxylin and eosin (H&E), toluidine blue, and Masson's trichrome (MTC) to examine the HP structure of the LGs[33].

2.6. Evaluating the effects of L-carnosine on LG apoptosis using IHC analysis of fatty acid synthase ligand (FasL)

The avidin-biotin-peroxidase complex method has been used to detect apoptotic changes in LG acinar cells (ACs) by localizing the antibody reaction against FasL, which induces apoptosis[34]. Sections from the LGs were deparaffinized, rehydrated, incubated in citrate buffer for antigen retrieval, and incubated in 3% H_2O_2, 5% bovine serum albumin, and unconjugated anti-FasL primary antibody at 4 °C overnight. The next day, the sections were incubated with a biotinylated secondary antibody and 3,3′-diaminobenzidine. Then, the sections were counterstained with Mayer's hematoxylin, and the reaction was visualized as a dark brown color in the cytoplasm of ACs. Negative control sections were obtained by omitting the primary antibody, while those of the positive control were obtained from the lymph node of the rabbit[35].

2.7. Morphometric analysis of the mean area percentage (%) of collagen fibers (CFs) and FasL in the LGs

Image analysis of data was performed on stored pictures from 10 random non-overlapping high power fields of sections from each rabbit of all groups. Pictures were acquired using a digital camera coupled to a light microscope, and the data were analyzed using Image J software 1.46 (NIH, Bethesda, MD, USA). The selected LG areas were determined on the image to measure the mean area percentage of CF deposition, and the cytoplasmic immunoexpression (CIE) of FasL in the acinar epithelium.

2.8. Biochemical measurement of serum IFN-γ, TNF-α, and TGF-β1 levels in all groups

Blood samples were collected from each rabbit, allowed to rest for 30 min, and centrifuged at 3000 rpm for 10 min to separate serum samples for the measurement of proinflammatory (IFN-γ and TNF-α) and profibrotic (TGF-β1) biomarkers. The samples were stored in Eppendorf tubes at –80 °C until assayed using a colorimetric assay kit, and absorbance was measured according to the manufacturer's instructions using an ELx800 absorbance microplate reader (Winooski, Vermont, USA) to measure the optical density (OD) at 450 nm.

2.9. Biochemical measurement of LG MDA and antioxidants

Small samples from the LGs of each rabbit were homogenized in isolation buffer (10 mM Tris, pH 8.0, and 1 mM ethylenediaminetetraacetic acid) and centrifuged at $3000 \times g$ for 20 min to obtain a supernatant from the LG homogenate[36]. MDA, SOD, GPx, and catalase levels were analyzed in the supernatant and, then, measured at their specific colorimetric wavelengths (695 nm, 450 nm, 340 nm, and 570 nm, respectively). All biomarker concentration calculations were evaluated according to the manufacturer's instructions and equations[37].

2.10. Determination of 2′,7′-dichlorofluorescin diacetate (DCFDA) cellular reactive oxygen species (ROS) in LG tissues using flow cytometry

Suspensions of single cells from the LGs of each rabbit were prepared to measure cellular ROS, according to Khan et al.[38,39].

2.11. Statistical analyses

All quantitative data were expressed as mean (M) ± standard deviation (SD) and were analyzed using SPSS 20 software (IBM, Chicago, IL, USA). One-way analysis of variance followed by Tukey's post-hoc test was used for intergroup comparisons. The data included the STT length, mean area percentage of CF deposition, CIE of FasL in the LGs, serum levels of IFN-γ, TNF-α, and TGF-β1, and supernatant levels of MDA, SOD, GPx, and catalase in the LG homogenate. Statistical significance was set at $P < 0.05$.

3. Results

3.1. Clinical effects of L-carnosine on an IL-1α-induced model of DED

The gradients of tear uptake (before and after the experiment) were measured using the STT to represent the extent of tear secretion in the LGs of all groups. The clinical results in Figure 1 reveal significant ($p < 0.05$) reductions in the STT of G2 rabbits compared with the normal STT gradients in G1 rabbits, indicating marked reductions in the secretory function of G2 LGs and the appearance of DED. In contrast, the STT values revealed significant improvements in the secretory functions in the LGs of G3 and G4 rabbits, indicating the anti-inflammatory effects of L-carnosine on the LGs of both groups compared with those of G2 rabbits.

3.2. Effects of IL-1α and L-carnosine on general LG structure

HP examination of the LG sections (stained with H&E; Figure 2) and semi-thin sections (stained with toluidine blue; Figure 3) exhibit numerous acini that consist of secretory ACs and myoepithelial cells (MECs) around an acinar lumen (ACL). G1 rabbits had a normal structure of the acini [formed of multiple ACs that lay on the basement membrane (BM) and MECs around the ACL], which were separated with thin loose areolar connective tissue (LACT). Conversely, the acini of G2 rabbits were thin, shrunken, and separated by inflammatory cells, congested blood vessels, marked edema, and thick LACT. Additionally, the cells were thin and had cytoplasmic vacuoles. These structural changes in G2 rabbits indicate marked inflammatory effects and disturbed LG secretory functions; these effects induced the features of DED after receiving IL-1α. In contrast, the acini of LGs in G3 and G4 rabbits revealed apparently normal acini with improved secretory functions, indicating the anti-inflammatory and antiapoptotic effects of L-carnosine on the LGs of G4 rabbits compared with those on G2 rabbits.

3.3. Effects of IL-1α and L-carnosine on CF deposition in the LGs

Examination of the LG sections stained with MTC revealed numerous acini around the ACL (Figure 4). G1 rabbits had a normal structure of the acini with a thin BM and LACT; however, G2 rabbits had areas of periacinar fibrosis with a thick BM and a thick interstitium of LACT, indicating the fibrotic effects of IL-1α on the LGs of G2 rabbits. In contrast, G3 and G4 rabbits showed an obvious improvement in the structure of their LGs with minimal periacinar fibrosis compared with that of G2 rabbits, indicating anti-inflammatory and antifibrotic effects of L-carnosine in G3 and G4 rabbits.

3.4. Effects of IL-1α and L-carnosine on the CIE of FasL in the ACs of LGs

As depicted in Figure 5, IHC examination of the LGs revealed negative CIE of FasL in the acini of G1 rabbits around the ACL, while G2 rabbits exhibited marked CIE of FasL in most acini, indicating the apoptotic effects of IL-1α in the ACs of G2 rabbits. In contrast, G3 and G4 rabbits showed negative CIE of FasL, except for a few acini that exhibited mild CIE of FasL around their ACL in G4 rabbits, indicating the marked improvement of AC structure owing to the anti-inflammatory and antiapoptotic effects of L-carnosine on the LGs of both groups.

3.5. Effects of IL-1α and L-carnosine on the morphometry of CFs and FasL in the LGs

The results from the morphometric image analysis shown in Figure 6 revealed significant increases in the mean area percentage of CF deposition and CIE of FasL in the LGs of G2 rabbits compared with those of G1, G3, and G4 rabbits. These results indicate the extent of fibrosis and apoptosis in the LGs, which led to dryness of the eye in G2 rabbits owing to the insufficient production of tears from their LGs. In contrast, the morphometric results indicated an obvious improvement in the LGs of G3 and G4 rabbits compared with G2 rabbits, owing to the beneficial anti-inflammatory, antiapoptotic, and antifibrotic effects of L-carnosine in both groups.

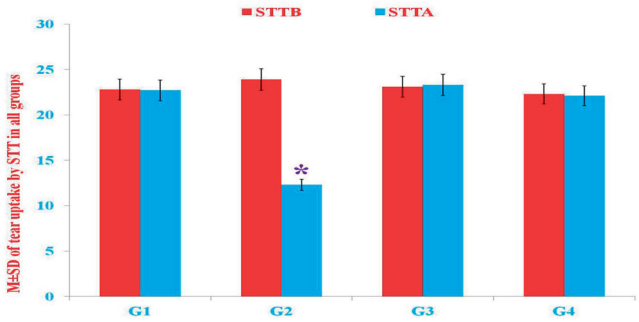

Figure 1. Clinical results of tear wetting length (mm/2 min) using a Schirmer tear test (STT) before (STTB), and after (STTA) the experiment in all groups. Values are expressed as the mean ± standard deviation (M ± SD, $n = 10$). *$p < 0.05$ denotes a significant difference when group 2 (G2) rabbits are compared to G1, G3, and G4 rabbits.

3.6. Effects of IL-1α and L-carnosine on serum levels of proinflammatory (IFN-γ and TNF-α) and profibrotic (TGF-β1) biomarkers

As shown in Figure 7, the serum levels of IFN-γ, TNF-α, and TGF-β1 demonstrate the effects of L-carnosine on the DED model. Serum levels of IFN-γ, TNF-α, and TGF-β1 were significantly increased in G2 rabbits compared with the normal levels in G1 rabbits, indicating the obvious proinflammatory and profibrotic effects of IL-1α, which caused DED in G2 rabbits. In contrast, these biomarkers significantly decreased in G3 and G4 rabbits compared to those in G2 rabbits, indicating the remarkable anti-inflammatory and antifibrotic effects of L-carnosine on the LGs of G3 and G4 rabbits.

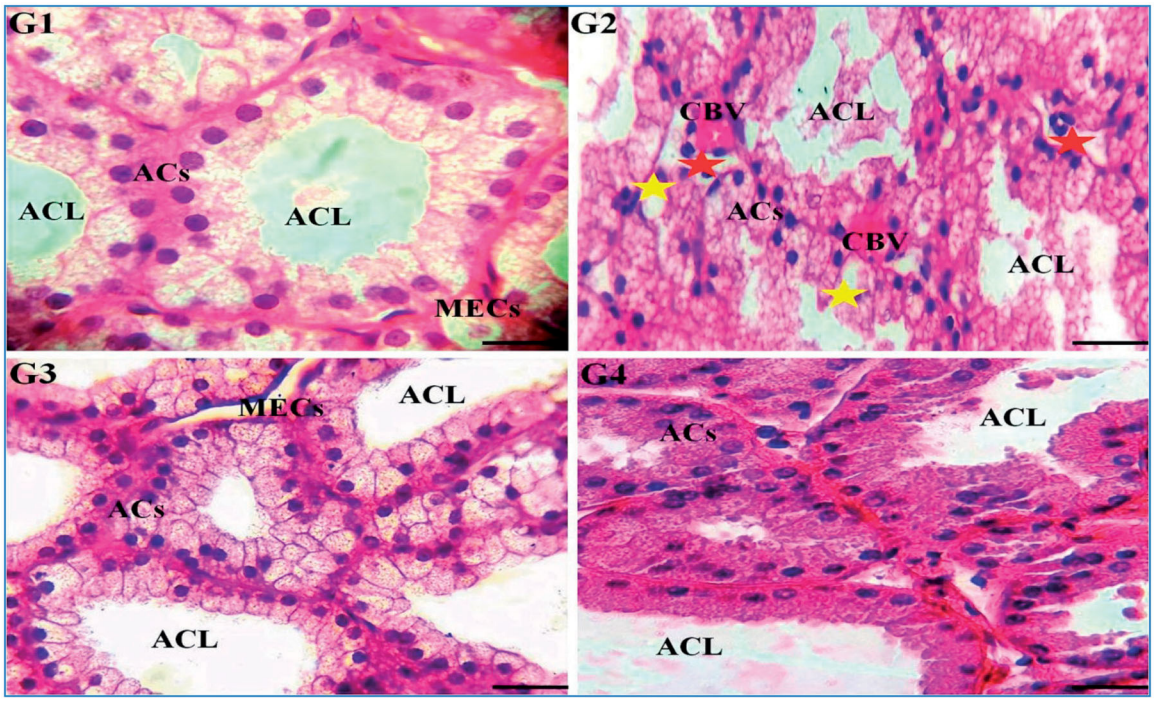

Figure 2. Photomicrographs from the lacrimal glands show numerous acini of acinar cells (ACs) and myoepithelial cells (MECs) around the acinar lumen (ACL). The group 1 (G1) rabbits have normal ACs around the ACL, while the acini of G2 rabbits are thin, shrunken, and the ACs have cytoplasmic vacuoles (yellow star). Additionally, the acini of G2 rabbits are separated by inflammatory cells (red star) and congested blood vessels (CBV). In contrast, the acini of G3 and G4 rabbits are similar to those of G1, and reveal normal ACs around the ACL. Hematoxylin and eosin staining, 630×, bar = 20 μm.

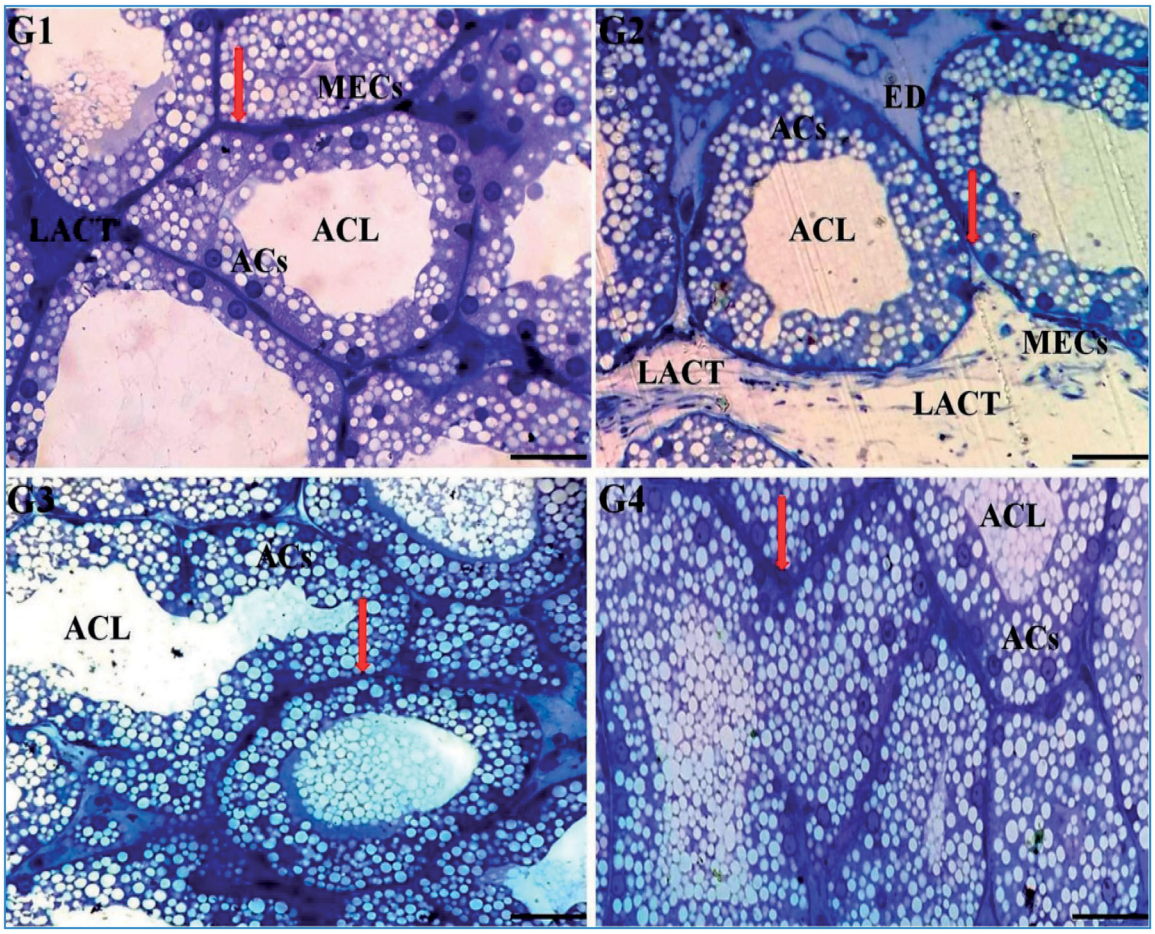

Figure 3. The lacrimal glands show numerous acini of acinar cells (ACs) around the acinar lumen (ACL). The acini of group 1 (G1) rabbits rest on a thin basement membrane (red arrow), and are surrounded by myoepithelial cells (MECs), and thin loose areolar connective tissue (LACT). In contrast, the acini of G2 rabbits have few secretions in their ACL, and are separated by marked edema (ED) and thick LACT, while the acini of G3 and G4 rabbits are similar to those of G1. Toluidine blue staining, 630×, bar = 20 µm.

3.7. Effects of IL-1α and L-carnosine on LG oxidative stress biomarkers

Biochemical measurements of the LG homogenate, as shown in Figure 8, exhibit significant increases in MDA levels and significant decreases in antioxidant levels (GPx, SOD, and catalase) in G2 rabbits compared with the normal levels in G1 rabbits. These remarkable changes in oxidative stress biomarkers in G2 rabbits indicate the existence of DED. Interestingly, G3 and G4 rabbits showed significant reductions in MDA levels with significant increases in the levels of the antioxidant biomarkers SOD, GPx, and catalase compared with those in G2 rabbits, indicating the powerful antioxidant effects of L-carnosine on the LGs of both groups.

3.8. Analysis of IL-1α and L-carnosine effects on cellular ROS of LGs using flow cytometry

As depicted in Figure 9, flow cytometry analysis from G1 rabbits revealed normal levels of DCFDA cellular ROS with a 7425 mean fluorescence index (MFI) in the LG homogenate, whereas it significantly increased to 23,753 MFI in G2 rabbits (inducing DED pathogenesis). In contrast, these levels were significantly reduced in G3 (7669 MFI) and G4 (12,137 MFI) rabbits compared with those in G2 rabbits, indicating the remarkable antioxidant effects of L-carnosine on the LGs of G3 and G4 rabbits.

4. Discussion

LGs are crucial for proper visual integrity, and their dysfunction usually plays a vital role in the initiation and progression of DED[40]. This disease affects millions of patients as a complication of chronic inflammation and ageing[41]. In the current study, adult male rabbits were chosen as an experimental model of DED to avoid any age or sex variations, because the LG function and disease are usually altered with variations in age and sex[42]. Herein, the effects of IL-1α on the LGs of rabbits and the possible cytoprotective effects of L-carnosine on the induced model of DED were investigated. Moreover, the effects of IL-1α and L-carnosine on LGs at the clinical, structural, and molecular levels were examined.

The quantitative analysis of clinical, HP, IHC, morphometric and biochemical evaluations of the LGs in G2 rabbits in this study, revealed significant reductions in the levels of STT gradient, marked diversity in the structure of LGs, periacinar fibrosis, and CIE of FasL in the ACs after treatment with IL-1α. These modifications are important indicators of marked

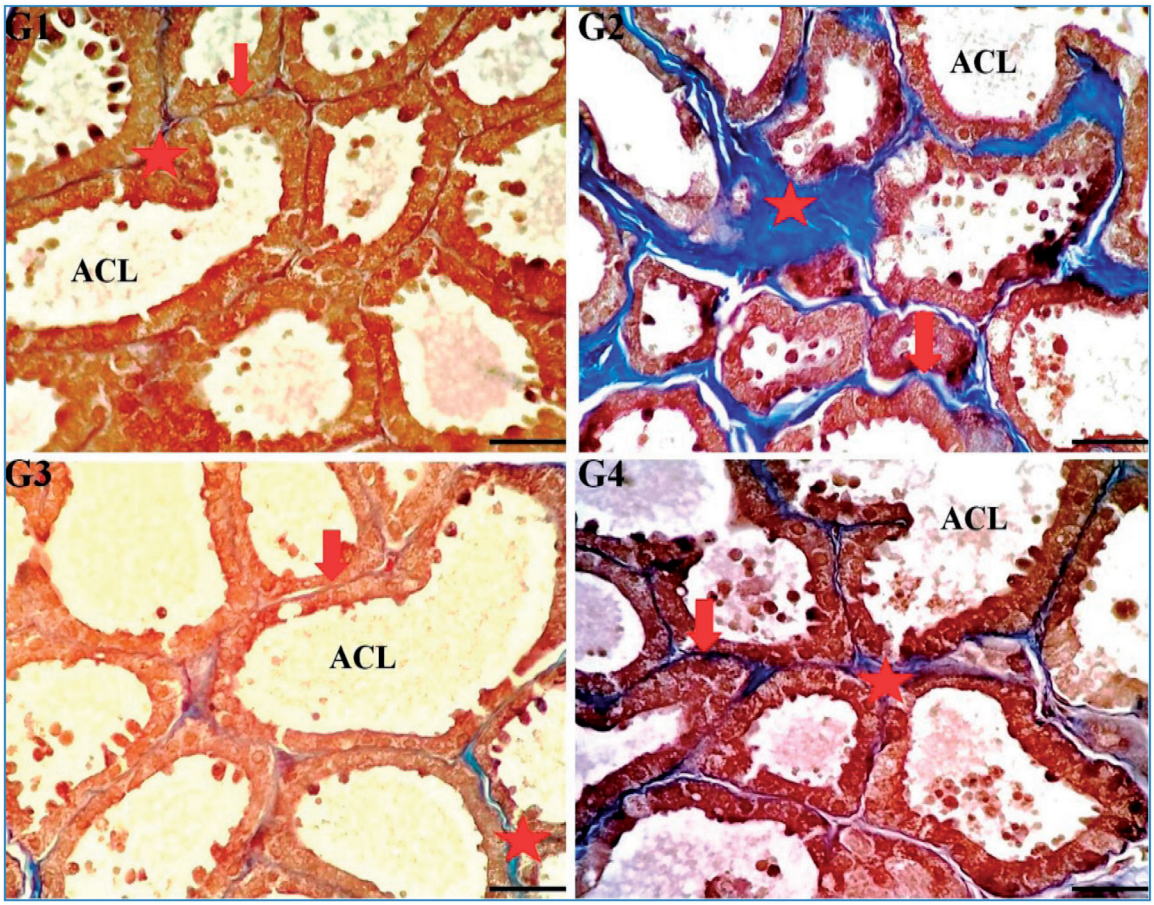

Figure 4. The lacrimal glands show numerous normal tubular acini around the acinar lumen (ACL) in group 1 (G1) rabbits, resting on a thin basement membrane (red arrow) and thin periacinar loose areolar connective tissue (red star), while the acini of G2 rabbits are separated by a thick basement membrane (red arrow) and periacinar fibrosis (red star). The acini of G3 and G4 rabbits are nearly similar to those of G1 rabbits. Masson's trichrome staining, 630×, bar = 20 μm.

inflammatory, apoptotic, fibrotic, and oxidative stress changes in the LGs, which reduce tear secretion and cause ADDED. In contrast, the results of G3 and G4 rabbits indicate powerful antioxidant, anti-inflammatory, antiapoptotic, and antifibrotic effects of L-carnosine on their LGs, which improve the production of tears and reduce the apoptotic, fibrotic, and oxidative stress changes in both groups owing to the mitigative antioxidant, anti-inflammatory, antiapoptotic, and antifibrotic effects of L-carnosine.

Collectively, these findings may be explained by a recent study that revealed LG impairment via induction of inflammatory responses to IL-1α, destruction of ACs, and reduction of aqueous tear secretion from the LGs[43]. In addition, oxidative stress and ROS generation inhibit the activity of antioxidant enzymes and cause apoptosis and damage to the LGs[30]. Noticeably, ROS induce multiple cell injuries, such as lipid peroxidation (LPO), oxidative membrane damage, and altered gene expression, which cause cell degeneration, FasL overexpression, and apoptosis in epithelial cells. These factors impair epithelial cell function and induce apoptosis in these cells[44]. Moreover, the imbalance between the production of free radicals and antioxidant radical scavenging systems is a major cause of oxidative stress and cell injury[45]. Apoptosis is enhanced by overexpression of FasL and cytochrome C (mitochondrial factors of apoptosis), which activate caspases and induce numerous apoptotic changes; in contrast, binding of FasL to Fas receptors in cases of Sjogren's syndrome induces apoptosis in Fas-expressing cells through the activation of caspase 3[36,46–48]. Furthermore, similar results to those of this study have been reported by Nebbioso et al., who state that the antioxidants SOD, GPx, and catalase are widely distributed in the mitochondria of ACs and are essential for normal secretion of tears[2]. SOD is the primary scavenging enzyme that catalyzes O_2 to H_2O_2 during antioxidative reactions, followed by the removal of H_2O_2 by catalase, resulting in the production of H_2O[49]. Additionally, the results of our study may be explained by Hejazi et al., who reported that accumulation of ROS in epithelial cells leads to extensive lipid oxidative stress and apoptotic changes. Conversely, other recent studies have demonstrated that FasL binds to its specific Fas receptors on the surface of epithelial cells and activate post-mitochondrial signaling pathways, triggering a series of caspases that induce apoptosis, which is very important for the removal of potentially dangerous cells[50–52]. Furthermore, the activation of these apoptotic signals impairs glandular secretory functions, whereas oxidative stress and overexpression of TGF-β1 lead to LACT thickening and AC shrinkage, which enhances LG atrophy[53]. In this context, the remarkable fibrosis and adhesions of the LGs are conferred mainly through loss of ACs, which induce expression of TGF-β1, delay

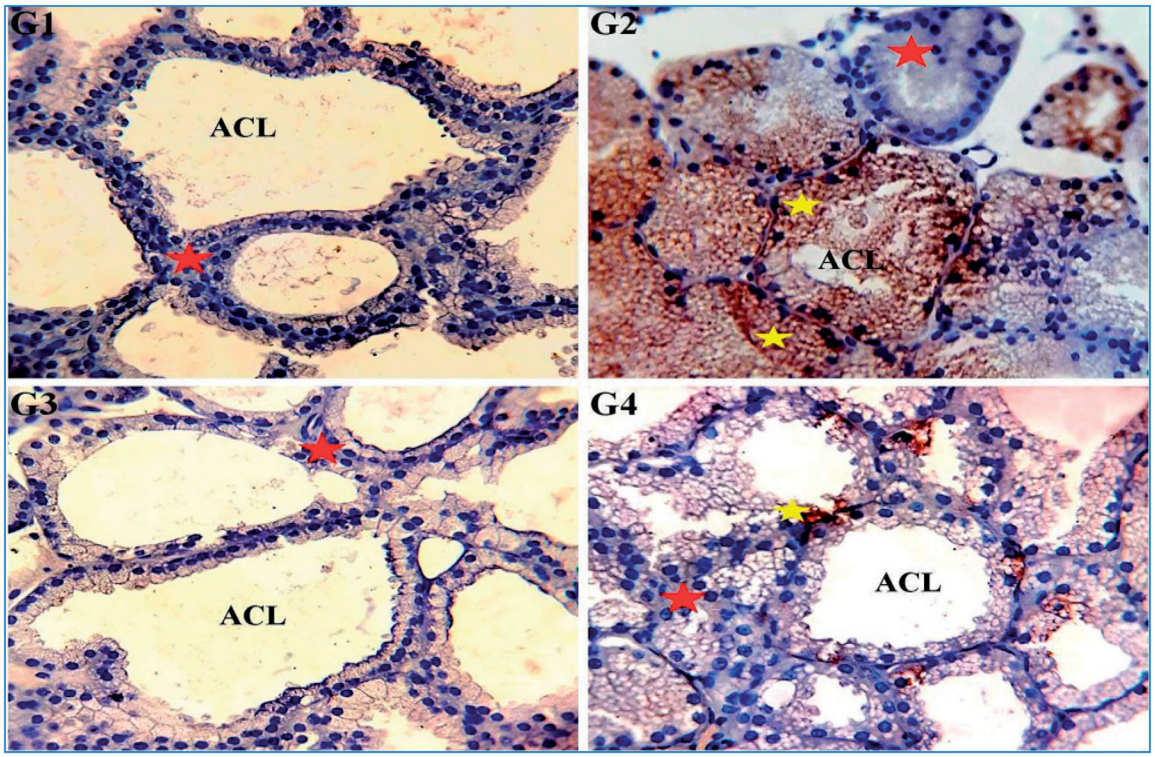

Figure 5. The acini of lacrimal glands show negative (red star) cytoplasmic immunoexpression (CIE) of fatty acid synthase ligand (FasL) around the acinar lumen (ACL) of group 1 (G1) rabbits, while the acini of G2 rabbits have a strong CIE of FasL (yellow star) in the majority of their acini with a weak CIE of FasL (red star) in some acini. In contrast, the acini of G3 and G4 rabbits show negative CIE of FasL (red star), while a few G4 acini exhibit a mild CIE of FasL (yellow star) around their ACL. FasL immunostaining, 630×, bar = 20 μm.

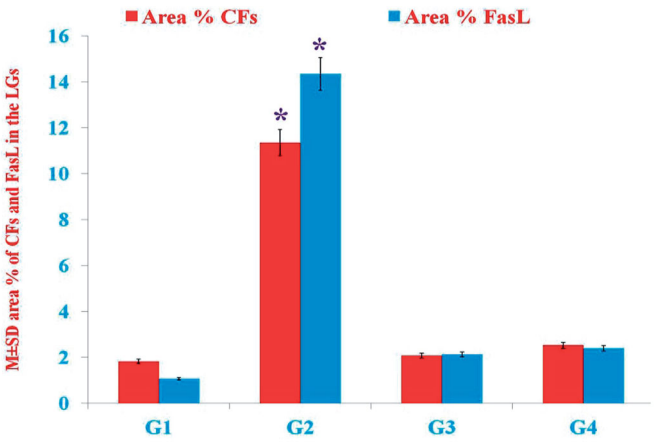

Figure 6. The mean area percentage (%) of collagen fiber (CF) deposition, and cytoplasmic immunoexpression (CIE) of fatty acid synthase ligand (FasL) in the lacrimal glands (LGs) of all groups. Values are expressed as the mean ± standard deviation (M ± SD, $n = 10$). $^{*}p < 0.05$ denotes a significant difference when group 2 (G2) rabbits are compared to G1, G3, and G4 rabbits.

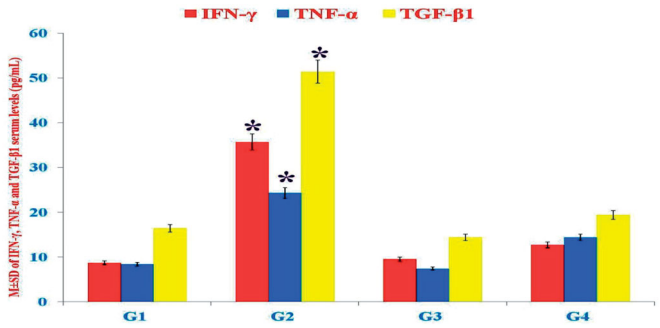

Figure 7. Blood serum measurements reveal significant increases in the levels of interferon (IFN)-γ, tumor necrosis factor (TNF)-α and transforming growth factor-β1 (TGF-β1) in group 2 (G2) rabbits compared to those in G1, G3, and G4 rabbits, while significant decreases of these levels are observed in G3 and G4 rabbits compared to those in G2 rabbits. Values are expressed as the mean ± standard deviation (M ± SD, $n = 10$). $^{*}p < 0.05$ denotes a significant difference when G2 rabbits are compared to G1, G3, and G4 rabbits.

reepithelialization, hinder regeneration, and enhance formation of the granulation tissue owing to reduction of collagenase enzyme production, deposition of CFs, and enhancement of fibrosis[54]. Interestingly, IL-1α-induced secretion of proinflammatory cytokines, such as IFN-γ and TNF-α, shorten and fragment AC microfilaments and delay exocytosis of the ACs[30,55]. From this perspective, these results may be explained by Toyama et al., who observed that dysfunction of the mitochondria is mediated by direct oxidative damage of the mitochondrial core proteins via activation of diverse pathways, such as the FasL/Fas system, which induce cell apoptosis[56]. In contrast, the results of G3 and G4 rabbits are supported by Davinelli et al., who note that L-carnosine contains numerous polyphenol molecules that prevent cellular damage via reducing ROS production and inhibiting the process of LPO[57,58]. Moreover, L-carnosine usage as a novel therapy for DED relies on its cytoprotective, antioxidant, anti-inflammatory, antiapoptotic, and antifibrotic effects on multiple organs against various free radical generating agents[59,60]. Indeed, pre-treatment with L-carnosine scavenges ROS free radicals and reduces epithelial cell damage via the inhibition of the LPO process of cell membranes, reducing proinflammatory (IFN-γ and TNF-α), profibrotic (TGF-β1), and MDA biomarkers, neutralizing free radicals, and restoring

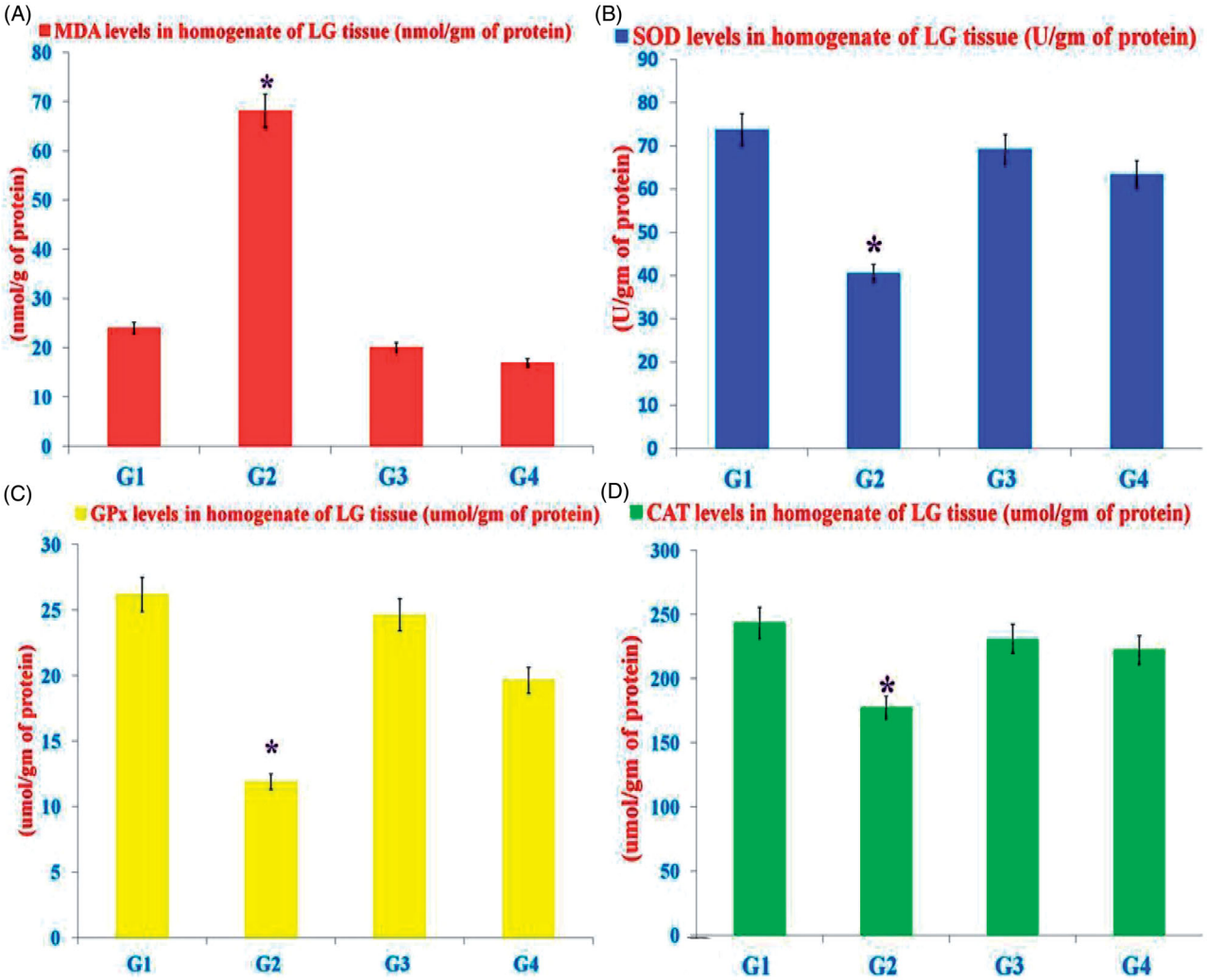

Figure 8. Biochemical measurements of the lacrimal gland (LG) homogenate in panel A reveals significant increases in malondialdehyde (MDA) levels in group 2 (G2) rabbits compared to those in G1, G3, and G4 rabbits. In contrast, panels B-D, respectively exhibit significant decreases in the levels of the antioxidant biomarkers (superoxide dismutase [SOD], glutathione peroxidase [GPx], and catalase [CAT]) in G2 rabbits compared to those in G1, G3, and G4 rabbits. Values are expressed the mean ± standard deviation ($n = 10$). $^{*}p < 0.05$ denotes significant a difference when G2 rabbits are compared to G1, G3, and G4 rabbits.

antioxidant enzyme levels (SOD, GPx, and catalase)[24,61]. Finally, L-carnosine reduces apoptotic signaling of FasL via inhibiting the release of numerous proinflammatory cytokines by macrophages[62].

5. Conclusion

Based on the results of this study, IL-1α exerted hazardous effects on the LGs, reduced their secretory functions, and led to dryness of the eye owing to its oxidative stress, proinflammatory, apoptotic, and profibrotic properties. In contrast, L-carnosine alleviated all previous effects via its antioxidant, anti-inflammatory, antiapoptotic, and antifibrotic effects. Therefore, intake of L-carnosine could be a novel adjuvant therapy to preserve the visual integrity of patients with DED. Finally, further studies are recommended for the possibility of L-carnosine application as eye drops to avoid the hazards of DED.

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Disclosure statement

The authors report no conflict of interest.

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Data availability statement

All relevant data have been provided in the manuscript.

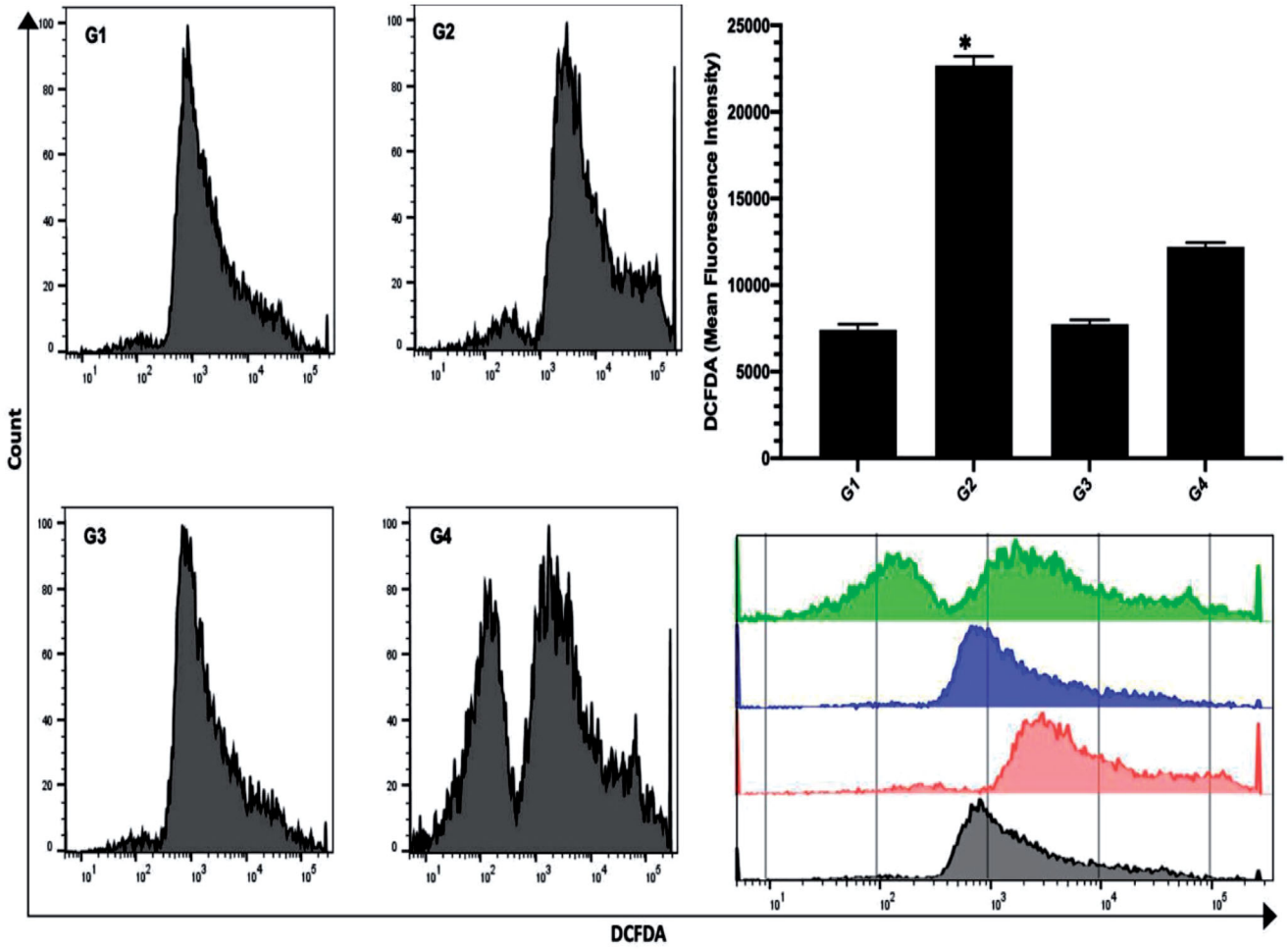

Figure 9. Cellular reactive oxygen species (ROS) measurements in lacrimal gland (LG) homogenates reveal significant increases in levels of 2′,7′-dichlorofluorescin diacetate (DCFDA) cellular ROS in G2 rabbits compared to those in the G1, G3, and G4 rabbits, while administration of L-carnosine exhibits significant reductions of these levels in G3 and G4 rabbits compared to those in G2 rabbits. Values are expressed as the mean ± standard deviation ($n = 10$). $^{*}p < 0.05$ denotes a significant difference when G2 rabbits are compared to G1, G3, and G4 rabbits.

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