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In vitro amoebicidal effects of arabinogalactan-based ophthalmic solution

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ABSTRACT

The main corneal infections reported worldwide are caused by bacteria and viruses but, recently, the number of *Acanthamoeba* keratitis (AK) cases has increased. *Acanthamoeba* genus is an opportunistic free living protozoa widely distributed in environmental and clinical sources, with two life-cycle stages: the trophozoite and the cyst. AK presents as primary symptoms eye redness, epithelial defects, photophobia and intense pain. An early diagnosis and an effective treatment are crucial to avoid blindness or eye removal but, so far, there is no established treatment to this corneal infection. Diverse research studies have reported the efficacy of commercialized eye drops and ophthalmic solutions against the two life cycle stages of *Acanthamoeba* strains, that usually present preservatives such as Propylene Glycol of Benzalkonium chloride (BAK). These compounds present toxic effects in corneal cells, favouring the inflammatory response in the so sensitive eye tissue. In the present work we have evaluated the efficacy of nine proprietary ophthalmic solutions with and without preservatives (ASDA Dry Eyes Eyedrops, Miren®, ODM5®, Ectodol®, Systane® Complete, Ocudox®, Matrix Ocular®, Alins® and Coqun®) against the two life cycle stages of three *Acanthamoeba* strains. Our work has demonstrated the high anti-*Acanthamoeba* activity of Matrix Ocular®, which induces the programmed cell death mechanisms in *Acanthamoeba* spp. trophozoites. The high efficacy and the absence of ocular toxic effects of Matrix Ocular®, evidences the use of the Arabinogalactan derivatives as a new source of anti-AK compounds.

1. Introduction

The eye is a complex organ with highly specialized constituent tissues derived from different primordial cell lineages (Hayashi, 2021). Currently, several corneal epithelial disorders such as oedema, blepharitis, glaucoma or the dry eye syndrome, represent the most common ophthalmic epithelial disorders (Seino et al., 2021). Different corneal infections are reported worldwide, mainly caused by bacteria but also by viruses and, as the most recent pathogenic microorganisms, opportunistic free living protozoa such as *Acanthamoeba* spp. (Oliveira-Ferreira et al., 2019). *Acanthamoeba* is widely distributed in environmental and clinical sources and presents two life-cycle stages: the vegetative form named trophozoite, and a high dormant resistant form or cyst (Siddiqui and Khan, 2012; Lorenzo-Morales et al., 2015).

The most common human pathology produced by this genus is the *Acanthamoeba* Keratitis (AK), that primary symptoms are eye redness, epithelial defects, photophobia and intense pain. An early diagnosis and an effective treatment are crucial to avoid blindness or eye removal (Lorenzo-Morales et al., 2015). Unfortunately, so far there is no established treatment for this corneal infection, but the recommended

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treatment regimen for AK includes a biguanide (0.02% polyhexamethylene biguanide (PHMB)/0.02% chlorhexidine digluconate) combined with diamidine (0.1% propamidine isethionate –Brolene-/0.1% hexamidine –Desomedine-) (Martín-Navarro et al., 2008, 2013). Recently, voriconazole has been demonstrated to be effective against different clinical strains of *Acanthamoeba* (Cabello-Vílchez et al., 2014; Rocha-Cabrera et al., 2015) and it has been successfully used in clinical cases too (Montiel et al., 2012).

Contact lenses wearers represent the highest risk group vulnerable to suffer AK, mainly due to the ocular traumas produced by the contact lenses or the lack of a correct hygiene (Lorenzo-Morales et al., 2015). Moreover, other corneal disorders such as the dry eye syndrome, is other disease derived from the use of contact lenses. Lately, diverse research studies have reported the efficacy of commercialized eye drops and ophthalmic solutions used for these other epithelial disorders, against the two life cycle stages of *Acanthamoeba* strains (Sifaoui et al., 2017, 2018, 2020a, b; Reyes-Batlle et al., 2019). Several commercialized ophthalmic solutions present preservatives such as Propylene Glycol, Benzalkonium chloride (BAK) or its derivatives such as POLYQUAD®, which have demonstrated antimicrobial (Kinnunen et al., 1991; Nalawade et al., 2015) or anti-*Acanthamoeba* activity (Sunada et al., 2014). However, both Propylene Glycol and BAK or its derivatives, induce inflammation on the ocular surface cells as cytotoxic effect (Paimela et al., 2012).

In the present work we have evaluated the efficacy of nine proprietary ophthalmic solutions (ASDA Dry Eyes Eyedrops, Miren®, ODM5®, Ectodol®, Systane® Complete, Ocudox®, Matrix Ocular®, Alins® and Coqun®) against the two life cycle phases of three *Acanthamoeba* strains, as well as the cell death mechanisms produced by the most active solution against *A. castellanii* Neff trophozoites. To the best of our knowledge, from the total of the evaluated solutions, only two of the contained preservatives. Furthermore, the most active eye drop, Matrix Ocular®, does not contain any preserving agent.

2. Material and methods

2.1. Chemicals

Nine eye drop solutions available commercially for topical use against DED were selected for analysis. Table 2 shows the details of the composition of these solutions.

2.2. Acanthamoeba spp. strains tested

The anti-*Acanthamoeba* activity of selected eye drops were initially evaluated against the trophozoite stage of *Acanthamoeba castellanii* Neff (ATCC 30010) type strain from the American Type Culture Collection. Subsequently, the most active eye drop solution was tested against two clinical isolates: *Acanthamoeba griffini*, genotype T3 obtained in a previous study (González-Robles et al., 2014) and *Acanthamoeba polyphaga,* genotype T4 (ATCC 30461). Those three strains were grown axenically in PYG medium (0.75% (w/v) proteose peptone, 0.75% (w/v) yeast extract and 1.5% (w/v) glucose) containing 40 μg gentamicin ml−1 (Biochrom AG, Cultek, Granollers, Barcelona, Spain).

2.3. In vitro effect against the trophozoite stage of Acanthamoeba spp.

The anti-*Acanthamoeba* activities of eye drop solutions were determined by the alamarBlue™ assay as previously described (McBride et al., 2005). Briefly, *Acanthamoeba* strains were seeded in duplicate on a 96-well microtiter plate with 50 μl from a stock solution of 2×10^4 cells ml−1. Amoebae were allowed to adhere for 15 min and 50 μl of serial dilution series of the eye drop solution were added. Finally, the alamarBlue™ Reagent (Life Technologies, Madrid, Spain) was added into each well at an amount equal to 10% of the medium volume. The plates were then incubated for 96 h at 26 °C with soft agitation. Finally, the plates were measured with the Enspire® microplate reader (PerkinElmer, Massachusetts, USA) using the emitted fluorescence (570/585 nm). Percentages of growth inhibition, 50% and 90% inhibitory concentrations (IC_{50} and IC_{90}) were calculated by non-linear regression analysis with 95% confidence limits. All experiments were performed three times, and the mean values were calculated. Differences between the values were assessed using one-way analysis of variance (ANOVA). Data are presented as means ± SD (N = 3); same letters indicate no significant differences when comparing different mean values of each eye drop.

2.4. In vitro effect against the cyst stage of A. castellanii Neff (ATCC300010)

The cysticidal activity was determined by the alamarBlue™ assay at 168 h and confirmed visually by inverted microscopy. *A. castellanii* Neff cysts were prepared as described by Lorenzo-Morales et al. (2008). Briefly, trophozoite were transferred from PYG medium based cultures (trophozoite medium) to Neff's encystment medium (NEM; 0.1 M KCl, 8 mM MgSO4·7H2O, 0.4 mM CaCl2·2H2O, 1 mM NaHCO3, 20 mM ammediol [2-amino-2-methyl-1,3-propanediol; Sigma Aldrich Chemistry Ltd., Madrid, Spain], pH 8.8, at 25 °C) and were cultured in this medium with gently shaking for a week in order to obtain mature cysts. After that, mature cysts were harvested and washed twice using PYG medium.

A serial dilution of the most active eye drop was prepared in PYG medium in sterile 96-well microtiter plates (NUNC, Thermo Scientific™). After this step, 5×10^4 *Acanthamoeba* spp. mature cysts ml−1 were added, obtained a final volume of 100 μL in each well. Plates were then incubated for 168 h at 26 °C with slight agitation as it has been described before (Sifaoui et al., 2018). After this initial incubation period, the 100 μL of each well were removed and added 100 μL of fresh PYG medium, in order to evaluate the excystation capacity of the treated cysts. Finally, 10 μL of the alamarBlue™ Reagent (Life Technologies, Madrid, Spain) was placed into each well, and the plates were then incubated for another 168 h at 26 °C with slight agitation. Subsequently, the plates were analysed, with an Enspire® microplate reader (PerkinElmer, Massachusetts, USA) using the emitted fluorescence (570/585 nm). Percentages of growth inhibition, 50% inhibitory concentration (IC_{50}) was calculated by linear regression analysis with 95% confidence limits. All experiments were performed three times each in duplicate, and the mean values were calculated.

2.5. Double-stain assay for programmed cell death determination

A double-stain apoptosis detection kit (Hoechst 33342/PI) (ThermoFisher™) and an inverted microscope EVOS FL Cell Imaging System (AMF4300, Life Technologies, USA) were used. The experiment was carried out by following the manufacturer's recommendations, and 10^5 cells ml−1 were incubated for 24 h with the previously calculated IC_{90}. The double-staining pattern allows the identification of three groups in a cellular population: dead cells with low-blue and high-red fluorescence (as the propidium Iodide stain enters the nucleus), live cells with low level or absence of fluorescence and cells developing PCD with a higher level of blue fluorescence.

2.6. Plasma membrane permeability

The SYTOX Green assay was performed to detect parasite's membrane permeability alterations. Initially, 10^5 ml−1 trophozoites were treated with the eye drop IC_{90} for 24 h. After this incubation, cells were washed and incubated in saline solution with SYTOX Green reagent (ThermoFischer™) at a final concentration of 1 μM for 15 min in darkness and following the manufacturer's instructions. Cells were observed and pictures were taken on the EVOS FL inverted microscope.

2.7. Analysis of mitochondrial membrane potential

The collapse of an electrochemical gradient across the mitochondrial membrane during apoptosis was measured using a JC-1 mitochondrial membrane potential detection kit (Cayman Chemical) as described by the manufacturer. Trophozoites treated with the IC_{90} of the evaluated eye drop were incubated for 24 h and then washed and resuspended in JC-1 buffer. 10 µL of JC-1 was added and incubated at 26 °C for 30 min in the darkness. Images were taken on the EVOS FL inverted microscope. The staining pattern allows to identify two different groups in the cellular population: live cells will show only red fluorescence, while cells with low mitochondrial potential (undergoing PCD) will show also red fluorescence and a higher level of green fluorescence.

2.8. Measurement of ATP

ATP level was measured using a CellTiter-Glo Luminescent Cell Viability Assay (ThermoFisher™) and following the manufacturer's recommendations. The effect of the evaluated eye drop on the ATP production was evaluated by incubating 10^5 of cells/ml with the previously calculated IC_{90} of the selected solution, adding the CellTiter-Glo reagent and measuring the luminescence in the Enspire® microplate reader.

2.9. Detection of reactive oxygen species (ROS)

During the apoptosis induction under both physiologic and pathologic conditions, reactive oxygen species play an important role. These species include a number of molecules that damage DNA and RNA and oxidize proteins and lipids. In order to detect ROS, we have used the CellROX® Deep Red Flow Cytometry Assay Kit. Treated cells were incubated with CellROX® at a final concentration of 5 µM for 30 min in darkness. Finally, those cells where the ROS have been produced, will show an intense red fluorescence. Images were taken on the EVOS FL inverted microscope.

3. Results

3.1. In vitro biological activity against Acanthamoeba spp.

From the 9 evaluated already commercialized eye drops, we would like to remark the *in vitro* efficacy of Matrix Ocular®, with the lowest IC50 value (7,09 ± 0.86%). Form the rest of the evaluated solutions, all of them have presented anti-*Acanthamoeba in vitro* activity with the exception of Ectodol® (Fig. 1). Among this other active eye drops, ASDA Dry Eyes and Ocudox® showed the highest inhibitory concentration (IC_{50}) values: 45,35 ± 9,66% and 45,14 ± 2.98%, respectively. However, the other six active compounds were able to inhibit the 50% of the *Acanthamoeba* population growth at concentrations lower than 20% (Fig. 1). As it could be observed in Fig. 1, there was no significant difference between Matrix Ocular® and ODM5®, Systane® Complete, Alins® and Coqun®. However, these last four compounds did not present a statistically significant difference with Miren either, which is significantly different to Matrix Ocular. Therefore, accordingly with these results, Matrix Ocular® was evaluated against the two different life cycle phases (trophozoites and cysts) of *A. castellanii* Neff and two clinical strains *A. polyphaga* and *A. griffini* (Table 1).

3.2. Evaluation of the mechanism of action of Matrix Ocular in A. castellanii Neff trophozoites

Regarding to the preliminary results, Matrix Ocular was chosen to continue the studies on the mechanism of action on the trophozoites of the three evaluated *Acanthamoeba* strains. Morphological events part of the PCD such as chromatin condensation, plasmatic membrane integrity, assessment of mitochondrial function disruption and the production of reactive oxygen species (ROS) (Kaczanowski et al., 2011) were evaluated as follow.

3.2.1. Double-stain assay for programmed cell death determination

In order to evidence the chromatin condensation, *A. castellanii* Neff (Fig. 2, B), *A. griffini* (D) and *A. polyphaga* (F) trophozoites were incubated with the IC_{90} of Matrix Ocular for 24 h and the double-stain Hoechst 33342/PI was performed. The intense blue and the scarce red fluorescence indicates an early phase of the apoptotic process, while the intense blue and red fluorescence evidences a late apoptosis process

Table 1
Inhibitory concentration 50 (IC_{50}) (%) of Matrix Ocular against the two different life cycle phases (trophozoites and cysts) of *A. castellanii* Neff, *A. polyphaga* and *A. griffini*.

Matrix Ocular®	Trophozoites IC_{50} (%)	Cysts IC_{50} (%)
A. castellanii Neff IC_{50} (%)	7,09 ± 0.86	8.93 ± 0.83
A. polyphaga IC_{50} (%)	7,51 ± 1.49	11.78 ± 0.12
A. griffini IC_{50} (%)	7,49 ± 0,66	11.66 ± 0.19

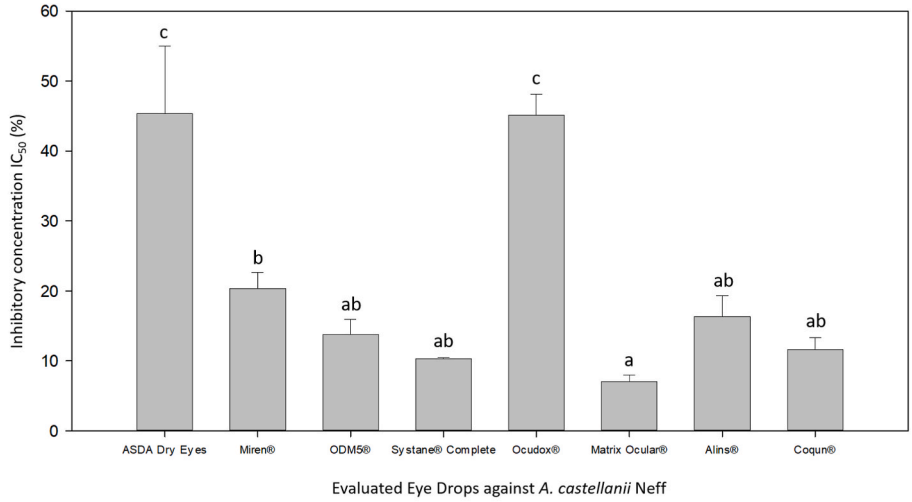

Fig. 1. Comparison of the amoebicidal effects of the 8 active commercialized eye drops against *Acanthamoeba castellanii* Neff trophozoites. Bars represent mean concentrations with each standard deviation. Differences between the values were assessed using one-way analysis of variance (ANOVA). Same letters indicate no significant differences when comparing different mean values of each eye drop.

Table 2
Detailed composition of the 9 commercialized solutions evaluated in the present study.

Eye Drop	Company	Components	Preservative
ASDA Dry Eyes Eyedrops	ASDA	Hyaluronic Acid 0.1%, Disodium Edetate	PHMB 0,0001%
Miren®	Brill Pharma	Riboflavin sodium phosphate, Vitamin E TPGS, Sodium Hyaluronate, MSM (methylsulfonylmethane), L-proline, L-glicine, chlorhydrate L-lisine, L-leucine, Sodium dibasic phosphate, Sodium chloride	No
ODM5®	Horus Pharma	Sodium chloride 5%, Sodium hyaluronate 0,15%	No
Ectodol®	Brill Pharma	Pentylene glycol, Glycerin, Ectoine, Polyglyceril-4 caprate, Glycerin glucoside, xanthan gum, panthenol, 4-terpineol, *Cananga ordorata* flower oil, Citrus lemon peel oil, Eugenol, Benzyl salicylate, geraniol, arnesol, linalool, benzyl benzoate, citral, limonene, water	No
Systane® Complete	Alcon	Boric acid, dimyristoyl phosphatidylglycerol, edetate disodium, hydroxypropyl guar, mineral oil, polyoxyl 40 stearate, sorbitan tristearate, sorbitol and purified water.	POLYQUAD® (polyquaternium-1) 0.001%
Ocudox®	Brill Pharma	Sodium chloride, Sosium phospate, Sodium sulphate, Sodium hypochlorite, Hypochlorous acid, Electrolysed water	No
Matrix Ocular®	Brill Pharma	Arabinogalactan; sodium tetraborate; boric acid, sodium chloride, water	No
Alins®	Brill Pharma	Sodium chloride 5%, injection water	No
Coqun®	VISUfarma	Co Enzyme Q10, vitamin E, Buffered isotonic solution	No

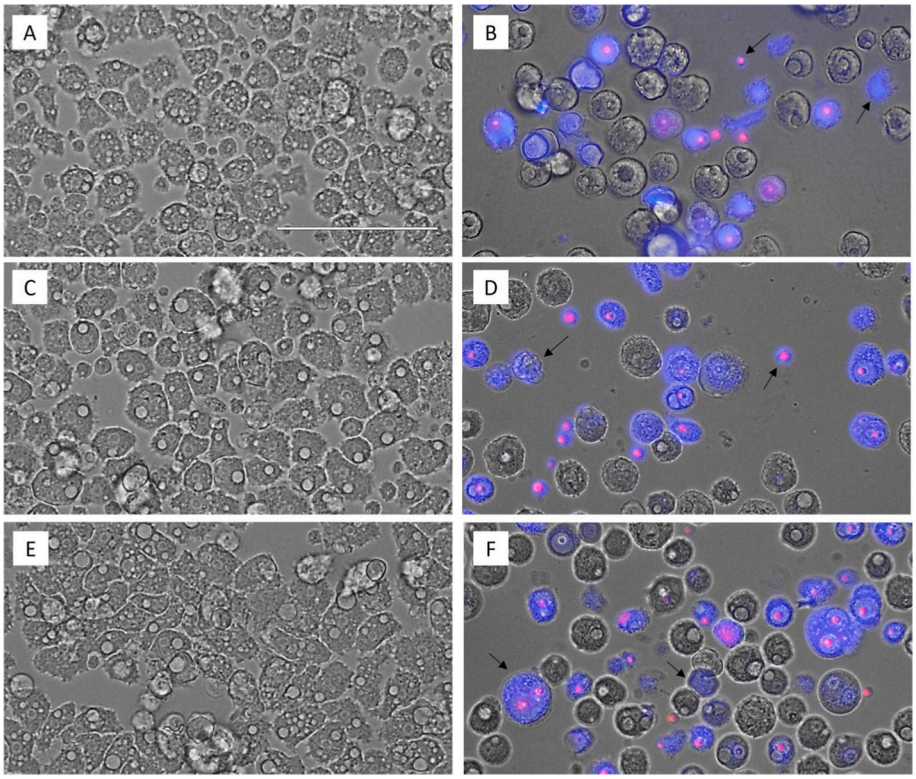

Fig. 2. Effect of the IC_{90} concentration of Matrix Ocular (B, D and F: *A. castellanii* Neff, *A. griffini* and *A. polyphaga* respectively) on the chromatin regarding the negative control (A, C and E, respectively) at 24 h. All images (40x) are based on Live Cell Imaging Microscope EVOS FL Cell Imaging System (Scale Bar 100 μm).

since the propidium iodide has reach to the nuclei material. *A. castellanii* Neff (Fig. 2, A), *A. griffini* (C) and *A. polyphaga* (E) trophozoites were incubated in fresh PYG medium as negative control, showing the total absence of fluorescence.

3.2.2. Plasma membrane permeability

An important evidence of the lack of a cellular necrotic process is the maintenance of the plasmatic membrane integrity. In Fig. 3, even though the plasmatic membrane permeability has been altered, the cell integrity was maintained, avoiding the emptying of the cellular content to the cytosol. The permeabilization of the *A. castellanii* Neff (Fig. 3, B), *A. griffini* (D) and *A. polyphaga* (F) trophozoites plasmatic membrane has been evidenced by the use of SYTOX® Green dye and it is due to the effect of the IC_{90} of Matrix Ocular after 24 h of incubation when comparing with each negative control (A, c and E, respectively).

3.2.3. Analysis of mitochondrial membrane potential

Fig. 4, shows that *A. castellanii* Neff, *A. griffini* and *A. polyphaga* trophozoites treated with IC_{90} of Matrix Ocular exhibit higher green fluorescence (B, D and F, respectively) comparing with each negative control (A, C and E). The green fluorescence indicates a decrease of the mitochondrial membrane potential, while in the negative control with an unaltered mitochondrial membrane potential we can observe red fluorescence caused by JC-1 dimers.

3.2.4. Measurement of ATP

The correct mitochondrial functioning depends on a suitable mitochondrial membrane potential, as well as an appropriate level of ATP. The measuring of the ATP level generated after the treatment with the IC_{90} Matrix Ocular for 24 h, showed a highly significant decrease compared to the negative control (Fig. 5). A One Way ANOVA test was

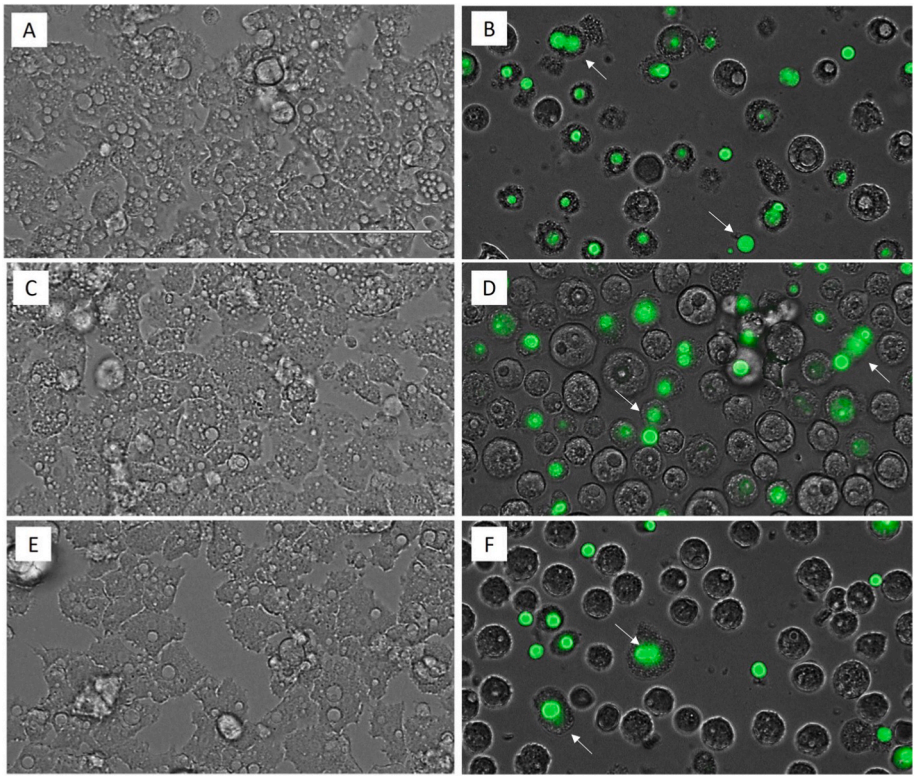

Fig. 3. Permeabilization of the *Acanthamoeba* spp. trophozoites plasmatic membrane evidenced by the SYTOX® Green dye due to the effect of the IC_{90} of Matrix Ocular (B, D and F: *A. castellanii* Neff, *A. griffini* and *A. polyphaga* respectively) in relation to the negative control (A, C and E, respectively) for 24 h. All images (40x) are based on Live Cell Imaging Microscope EVOS FL Cell Imaging System (Scale Bar 100 μm). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

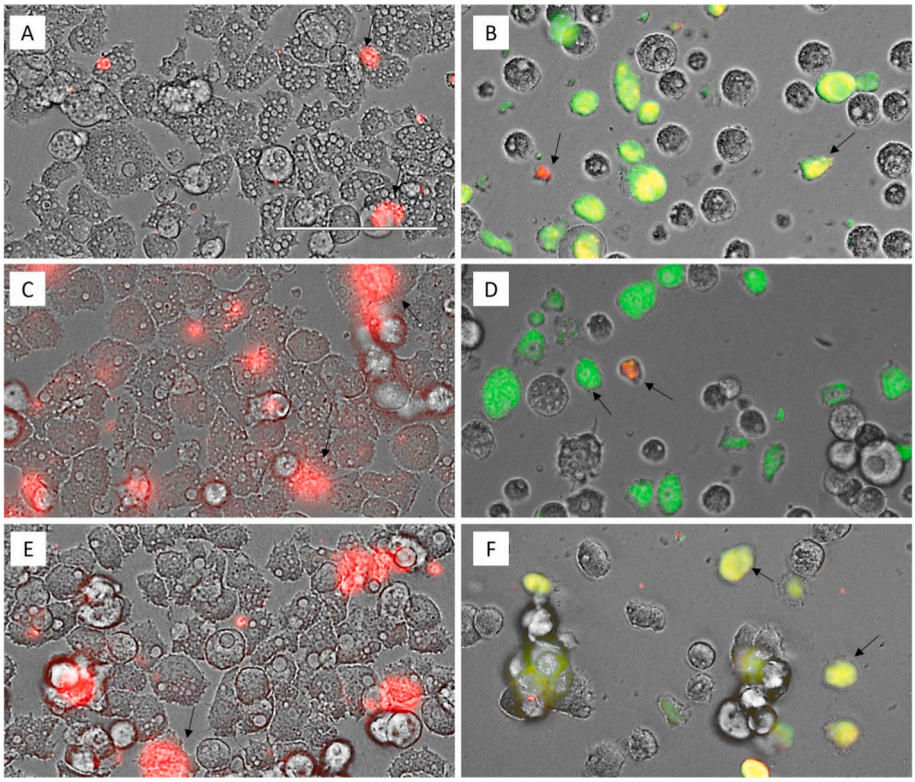

Fig. 4. Effect on the mitochondrial potential evidenced by the JC-1 assay kit in trophozoites treated with the IC_{90} of Matrix Ocular (B, D and F: *A. castellanii* Neff, *A. griffini* and *A. polyphaga* respectively) in relation to the negative control (A, C and E, respectively) for 24 h. All images (40x) are based on Live Cell Imaging Microscope EVOS FL Cell Imaging System (Scale Bar 100 μm).

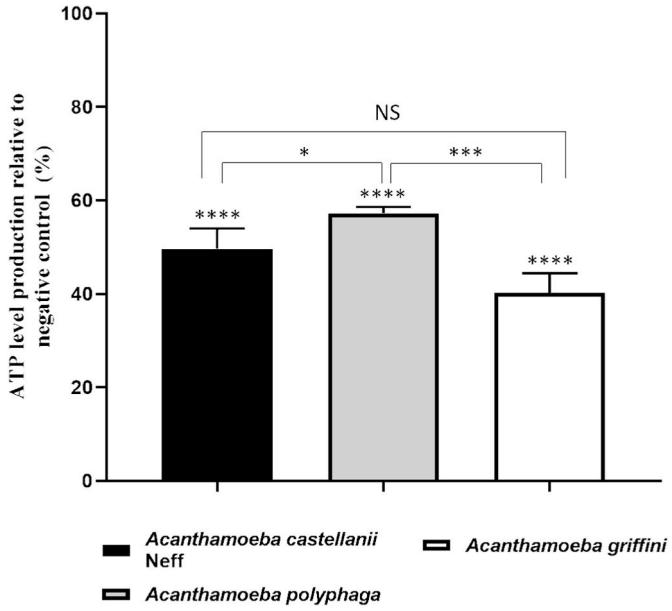

Fig. 5. ATP level decreasing in the three evaluated *Acanthamoeba* spp. A One Way ANOVA test was carried out to test the statistical differences between means. (NS [non significance]); $p < 0.05$ [*]; $p < 0.001$ [***]; $p < 0.0001$ [****]).

carried out to test the statistical differences between means, $p < 0.0001$ (****). Moreover, while there were significantly differences between *A. castellanii* Neff and *A. polyphaga*, $p < 0.05$ (*) and *A. polyphaga* and *A. griffini*, $p < 0.001$ (***), there was no difference between *A. castellanii* Neff and *A. griffini*. This last strain has demonstrated to present the most affected ATP level due to the effect of Matrix Ocular (<40% when comparing to the negative control).

3.2.5. Detection of reactive oxygen species (ROS)

The staining with the CellROX® Deep Red has demonstrated that the trophozoite treatment with IC90 of Matrix Ocular generated intracellular ROS after 24 h in the three evaluated strains (Fig. 6).

4. Discussion

The number of *Acanthamoeba* Keratitis (AK) cases has increased due to contact lens wearers rise in both developed and developing countries (Maycock and Jayaswal, 2016; Brown et al., 2018). Even though this protozoa infection is rare in the general population (estimated incidence: 1.4 cases/million people/year), it is highly frequent in contact lens wearers population (Khan 2009; Trabelsi et al., 2012; Lorenzo-Morales et al., 2015). An early and effective treatment is crucial to avoid side effects such as blindness or eye removal (Lorenzo-Morales et al., 2015). One of the most common problems in the AK treatment is the cyst form of *Acanthamoeba*, which is highly resistant to therapy. Therefore, a long-term treatment with different drug combinations is necessary to remove both cysts and trophozoites (Lim et al., 2008; Mirjalali et al., 2013). Despite of the recommended treatment regimen for AK that includes a biguanides such as chlorhexidine (Martín-Navarro et al., 2008, 2013), there is no totally effective treatment against AK until now. Most of the already commercialized ophthalmic solutions present different preservatives such as Propylene Glycol or benzalkonium chloride (BAK), whose have demonstrated to be effective against the two life cycle stages of *Acanthamoeba* spp. (Sifaoui et al., 2017, 2018). Nevertheless, it is important to highlight that the AK most common treatment Chlorhexidine, presents a higher toxicity in corneal cells than the BAK (Sapozhnikov et al., 2020).

In the present study we have evaluated the anti-*Acanthamoeba* effect of nine different ophthalmic solutions. Only ASDA Dry Eyes and

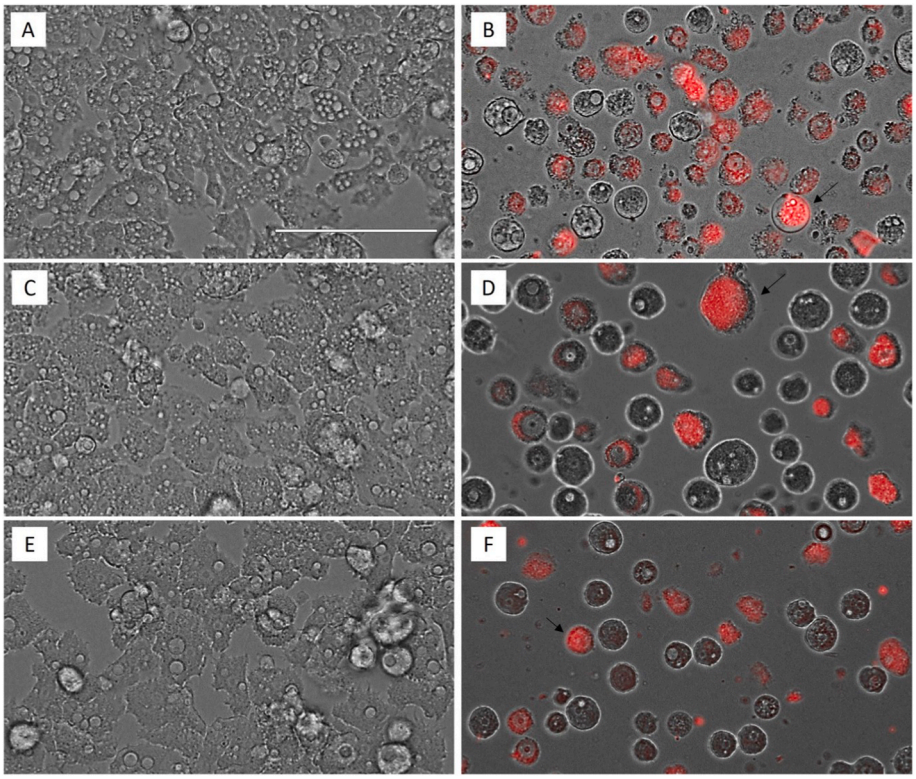

Fig. 6. Detection of reactive oxygen species (ROS) in trophozoites treated with the IC_{90} of Matrix Ocular (B, D and F: *A. castellanii* Neff, *A. griffini* and *A. polyphaga* respectively) in relation to the negative control (A, C and E, respectively) for 24 h. All images (40x) are based on Live Cell Imaging Microscope EVOS FL Cell Imaging System (Scale Bar 100 μm).

Systane® Complete present preservatives (PHMB 0,0001% and POLYQUAD® (polyquaternium-1) 0.001%, respectively) (Table 2). However, the other seven analysed compounds do not present any preservative. Interestingly, ASDA Dry Eyes has presented the highest IC_{50}, only preceded by Ectodol®, which was totally ineffective against *A. castellanii* Neff since the recommended concentration of PHMB to treat AK is 0.02% (Martín-Navarro et al., 2008, 2013). On the other hand, Systane® Complete contains 0.001% of POLYQUAD®, which has demonstrated to damage *A. castellanii* trophozoites and cysts at 0.05% and 0.005% of BAK (Sunada et al., 2014). However, in 1998 Niszl and Markus, presented a study where they demonstrated that 0.004% of BAK results inefficacious against *Acanthamoeba* cysts (Niszl and Markus, 1998). Therefore, taking into account these previous studies, our work remark the Systane® Complete anti-*Acanthamoeba* activity, independently of the BAK concentration.

Related to the other seven ophthalmic solutions without preservatives, Matrix Ocular® demonstrated to be the most effective against *A. castellanii* Neff trophozoites. Thus, we tested its efficacy against the two life cycle phases of *A. castellanii, A. polyphaga* and *A. griffini,* as well as to evaluate the induction of cell death mechanisms in these strains (Table 1). While the IC_{50} values against the trophozoite stage are similar in the three tested strains, *A. castellanii* Neff presented the lowest IC_{50} value against cysts, becoming the least resistant strain. Both *A. polyphaga* and *A. griffini* clinical strains have presented similar IC_{50} against the cyst stage (Table 1). There are different reports whose demonstrate the efficacy of the Chlorhexidine against these two *Acanthamoeba* stages (Martín-Navarro et al., 2008, 2013) and despite it has been described as one of the most effective treatments against AK (Lorenzo-Morales et al., 2015), we have to highlight that Chlorhexidine presents a high toxicity in corneal cells (Sapozhnikov et al., 2020). Matrix Ocular® is an already commercialized ophthalmic solution with a lubrication effect as main purpose and it is medically prescribed to protect the cornea and the conjunctiva after surgeries or epithelial damage. Matrix Ocular® has been developed from a natural polysaccharide named Arabinogalactan, which has been isolated from the wood of the softwood plant *Larix* spp. This genus can be found widely distributed across regions of China, Russia, Canada, Central Europe, and other cool, temperate regions of the northern hemisphere (Mason and Zhu, 2014). Arabinogalactan (5% w/w) has been reported as a novel mucoadhesive polysaccharide which can be used for the treatment of dry eyes and corneal wounds and to heal the dry spots on cornea (Burgalassi et al., 2007). Moreover, as it has been commented previously, Matrix Ocular® does not present any preservatives, whose could present toxic effects in corneal cells, such as Propylene Glycol or BAK (Sapozhnikov et al., 2020). Novel polymers, like arabinogalactan, have demonstrated the potential to safely deliver drugs at a controlled rate in different ophthalmic formulations (Pahuja et al., 2012). A recent study has demonstrated the beneficial effect of the synergetic formulation of Arabinogalactan and hyaluronic acid on the mitigation of the inflammatory process in the worsening of dry eye syndrome (Silvani et al., 2020). Besides, in the present research we have evidenced the programmed cell death induced by Matrix Ocular®. The absence of a necrotic cell death to avoid an excessive inflammatory response in the host, is one of the key to develop new therapies against *Acanthamoeba* infections. Additionally, there is a need to increasing the search of new molecules which produce programmed cell death (PCD) or apoptosis-like processes. Therefore, taking into account this fact and the mitigation effects of the inflammatory process in the dry eye syndrome, our study proposes Matrix Ocular® to continue with further studies to elucidate new treatments against AK.

5. Conclussions

In the present work, we have demonstrated that Matrix Ocular® is effective against the two life cycle stages of *Acanthamoeba* spp., inducing programmed cell death mechanisms in the three evaluated pathogenic strains. Therefore, Matrix Ocular® is an interesting starting point for further studies in order to develop a novel and effective treatment against AK, with an important mitigation of the inflammatory process.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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