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Research Article

Therapeutic Effect DMSO, Pentostam and Chloramphenicol on Mice Eye Infection with Amoebic Keratitis

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Abstract:
The current study proved that the Dimethylsulfoxide (DAMSO) compound caused the healing of the eyes of animals infected with Acanthamoeba, but caused scratching of the corneas of the experimental animals, while Cisplatin treatment had no effect on the parasite and caused severe corneal necrosis, and the Chloramphenicol did not show any therapeutic effect on the parasite.

Keywords: dimethylsulfoxide, acanthamoeba, cisplatin

INTRODUCTION

The cornea, or transparent portion of the front of the eye, becomes infected when amoebae of the genus Acanthamoeba invade it. This rare illness is called Acanthamoeba keratitis (AK). It affects around one hundred Americans a year. In 2019 Scruggs et al. The skin, eyes, and central nervous system can all become infected with ameba, a kind of protozoa that is virtually always found in soil and water. [1] There are some species of *Acanthamoeba* that can cause Acanthamoeba keratitis, such as *Acanthamoeba castellanii, A. polyphaga and A. quina [2]*. The most typical way that Acanthamoeba spp. enter the eye is through contact lenses that have come into touch with the organism. This can happen when using contaminated lens solution, when using homemade saline-based solution or tap water, or while wearing contact lenses while taking a bath or swimming. AK disease regularly has effects on the individual who not sterile their contact lenses, also affects the people who are bathing or swimming while wearing contact lenses[3]. In addition, this infection might happen in individuals who do not use contact lenses by dealing with contaminated water and soil with *Acanthamoeba* But it can also enter the eye from trauma or contact with dirt or plants. [4]The infected and development of AK might take a few days to several weeks, depending on the way of acanthamoeba's entrance. For example the infection development happened by using contaminated contact lenses is a slower than the case of corneal trauma[5].

Once a contact lens is in place, Acanthamoeba may reside in the space between the lens and the eye's surface.[6] Acanthamoeba can attach itself to mannosylated glycoproteins on the surface of the cornea because soft contact lenses adhere to the cornea's surface more firmly than hard lenses do.[7] Wearing contact lenses increases the expression of these proteins on the corneal surface. [6] The conjunctiva being coated with secretory IgA prevents adhesion oftrophozoites to corneal epithelial cells. Previous studies have been showed that the method of antiAcanthamoeba IgA antibodies to protect against AK via inhibiting the trophozoites adherence to corneal epithelial cells without affecting the viability of trophozoites of *Acanthamoeba[8]*.

While most cases of Acanthamoeba keratitis are associated with contact lens wearers, numerous non-wearers have also been recorded cases of the parasite, especially outside of the US.[9] For non-contact lens wearers, the most common causes of Acanthamoeba infection are trauma and contaminated water exposure. [10] he biguanides, which include chlorhexidine (0.02 to 0.2% drops) and polyhexamethylene biguanide (PHMB) (0.02% to 0.06% drops), are one type of drugs used in therapy.
[11]The trophozoite organism dies as a result of these drugs damaging its cell wall. These medications, however, haven't shown to be very effective against the cystic types.[10] These treatments are frequently used as the first line of

treatment for AK because of their effectiveness against Acanthamoeba and their little toxicity to the cornea.**[7]**

The aim of the study: we found that DMSO has an inhibitory effect on the *Acanthamoeba* in in vitro . Therefore, the current study aimed to determine its efficiency as a treatment for eye infection with the *Acanthamoeba tringularis* and to test iodine and Pentostam as a sterilizing agent for similar infections.

MATERIALS AND METHODS

The parasite was collected from the urine of a patient with kidney failure and classified as *Acanthamoeba triangularis* ,the parasite was cultivated in the laboratory on Non-nutrient agar medium, then it was transferred to agar medium with sheep brain to activate it for a period of four days.

The parasite was then collected from the culture media using test tubes and washed several times with sterile fresh water using a centrifuge .

Infection laboratory animals: 12 mice Balb/C bread in a special cage. They were fed and provided with water. They were then anesthetized by injecting them with a mixture of xylazine and ketamine 1-2 to facilitate the infection of the eyes with the parasite. The animals were left until symptoms of infection appeared, after which they were treated as follows.

2 mice were treated with 0.1 ml of DMSO
2 mice were treated with 0.1 m iodine
2 mice were treated with 0.1 ml pentostam
2 mice left untreated
And 2 mice were not injured control group eye treatment occur as eye drop

The mice treated daily for seven day , at the end of the treatment period, the animals were dissected and mice eyes were removed a fixative with 10% formalin for 24 hours, the specimen dehydrated with ethyl alcohol 70%, 90% two hours for each concentration, and 100% was repeated twice, an hour for each.

Then it was transferred to xylene clearing agent five hours, then placed in molted paraffin wax for six hours. The wax was replaced every three hours.

Then embedded. The paraffin block sectioned by rotary microtome at 6-7 microns thickness and stained with hematoxylin-eosin, examined and photographed.

RESULTS

The color of the eyes of laboratory mice tends to be bright red (Figure 1A), while infection with Acanthamoeba causes a change in eye color cloudy white area, (Figure 1B). a cross-section of the whole eye showed its components the cornea, lens, and retina(Figure 2). The current study showed the ability of the *Acanthamoeba triangularis* to infect the epithelial layer of the eyes of untreated mice, causing tissue necrosis (Figure 3). It was also possible to observe the parasite in the parasite invade optic nerve also (Figure 4). The tissue sections of the animals' eyes did not show any infection in the retina (Figure 5).It was also observed that the parasite reached the lacrimal glands, and the presence of the parasite in its duct was recorded (Figure 6). The mice eyes Treated with DMSO cause Corneal detachment ,in addition to the parasite not observed in the tissue (Figures 7 and 8), and the parasite was not observed in the optic nerve area, (Figure 9). The treatment of eyes with Cisplatin caused severe necrosis of the cornea but the parasite not effected by the treatment , the parasite appeared in the area of the cornea with bleeding and necrosis (Figure 10). Corneal necrosis was also observed in other instances, (Figure 11). Inflammatory response were also observed in the deep areas of the cornea large number of inflammetry cells was found, (Figure 12).

Chloramphenicol treatment was not show any effect on eyes infection , the parasite observed in the corneal area, with bleeding and tissue necrosis , (Figure 13).

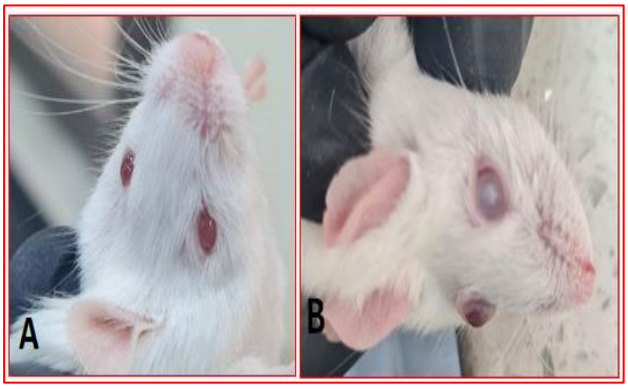

Figure 1) **A** -The color of the eyes of laboratory mice tends to be bright red , B- while infection with *Acanthamoeba* causes a change in eye color to a cloudy white .

Figure 2) eye infected with the *Acanthamoeba triangularis* cornia red arrow, Iris yellow Arrow, Lens black arrow and retina blue arrow .

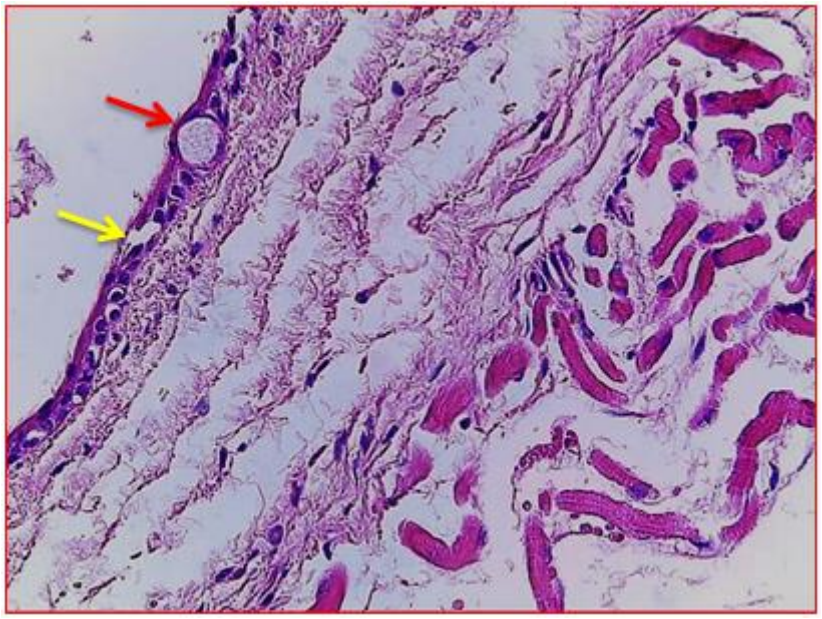

Figure 3) eye infected with the *Acanthamoeba triangularis* the parasite infect epithelial tissue red arrow, and tissue necrosis yellow Arrow.

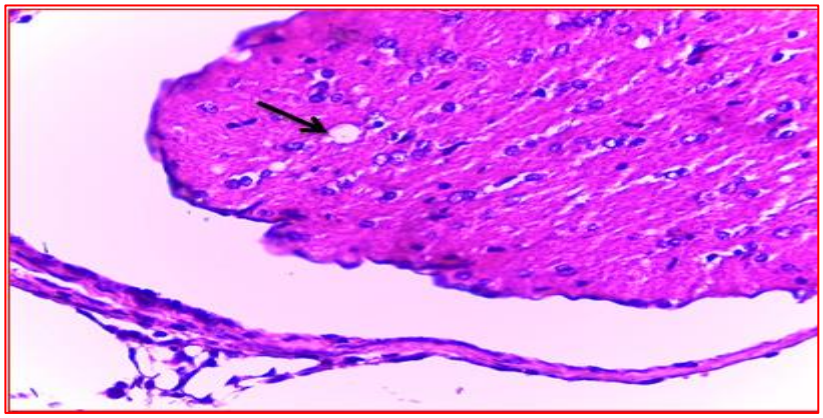

Figure 4) parasite in optic nerve of an infected untreated mouse showing the presence parasite ,arrow

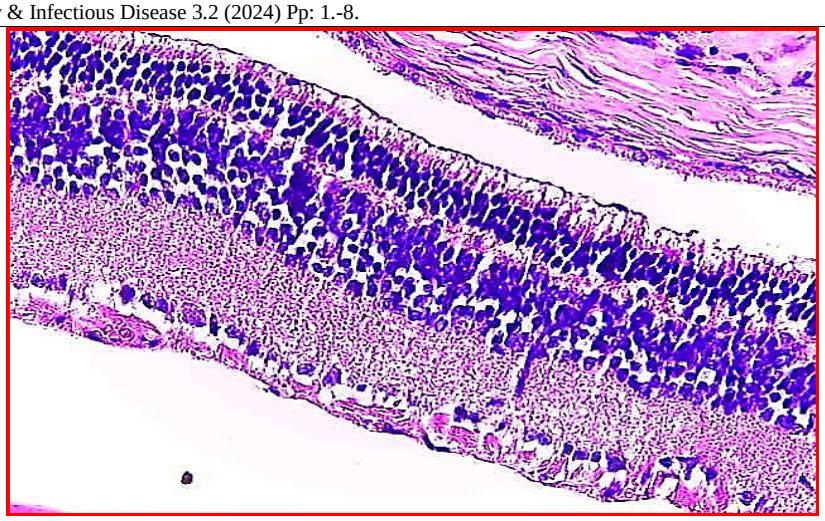

Figure 5) The retina of an infected untreated mice, the parasite not reached the retina

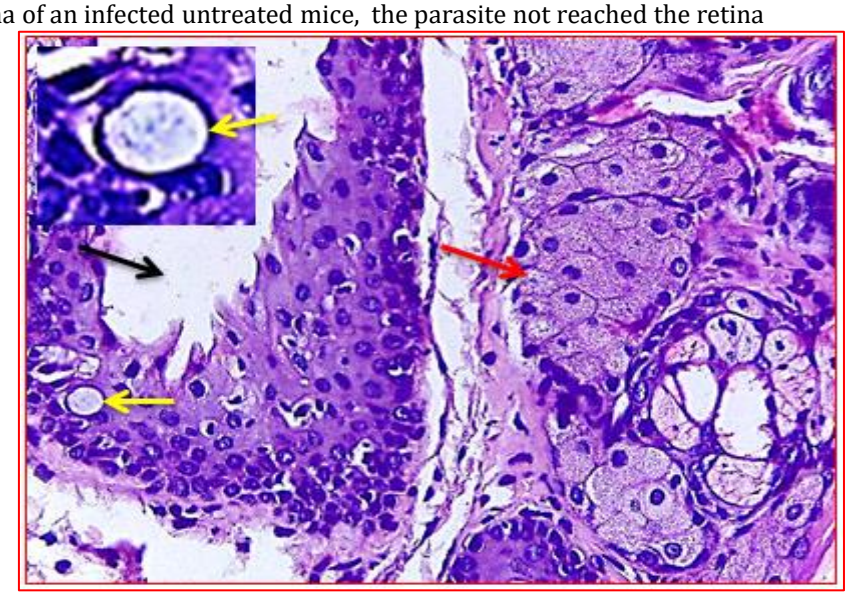

Figure 6) show the parasite yellow arrow reached the lacrimal gland red arrow in the duct black arrow .

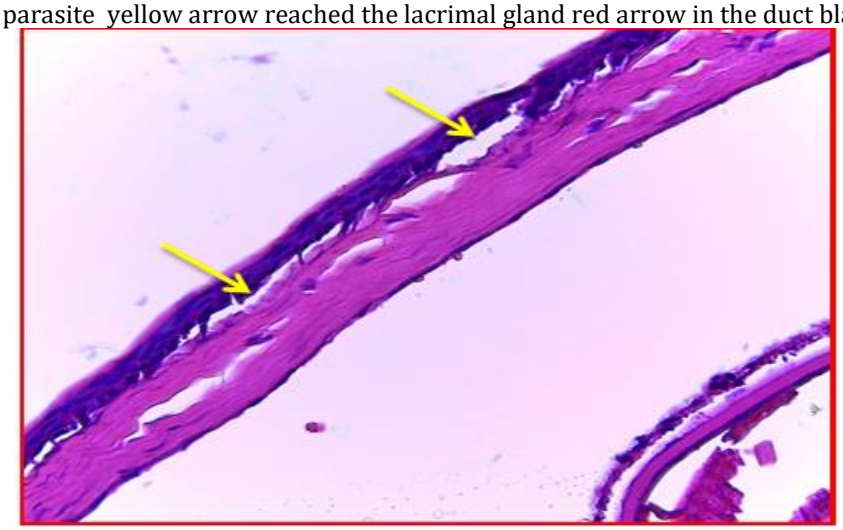

Figure 7) The mice eyes Treated with DMSO show Corneal detachment arrow the parasite was not seen.

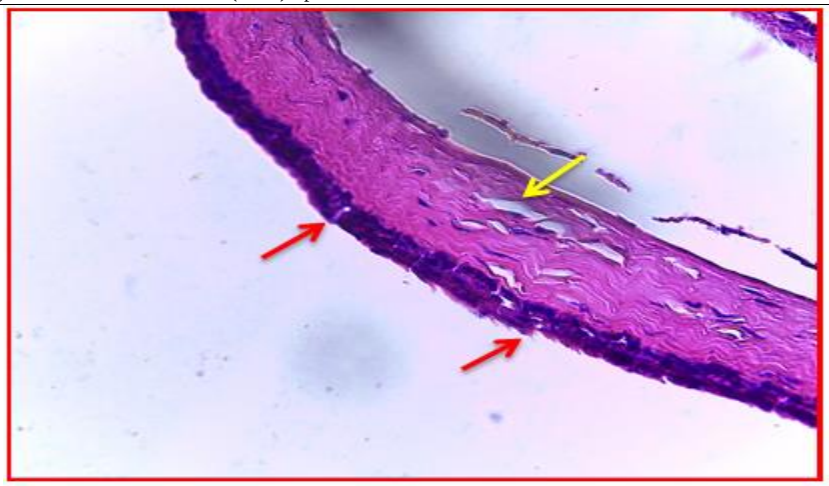

Figure 8) The mice eyes Treated with DMSO show Corneal detachment yellow arrow and epithelial cell necrosis red arrow the parasite was not seen.

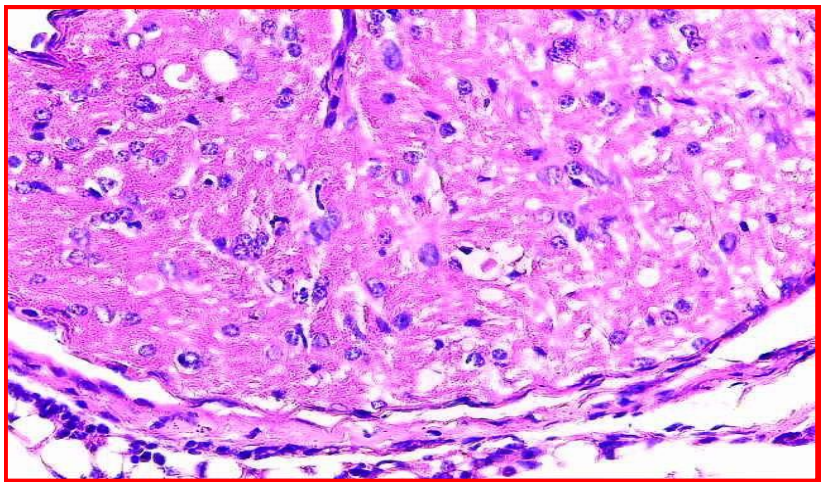

Figure 9) The parasite not observed in the optic nerve .

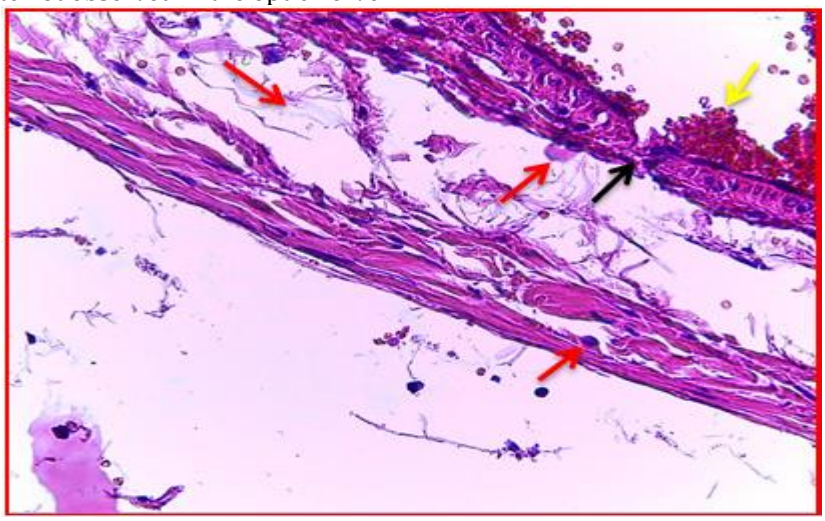

Figure 10) A section of the eye of infected mice treated with Cisplatin chemotherapy , showing the presence of the parasite, red arrow bleeding yellow arrow and necrosis of the corneal tissue, black arrow.

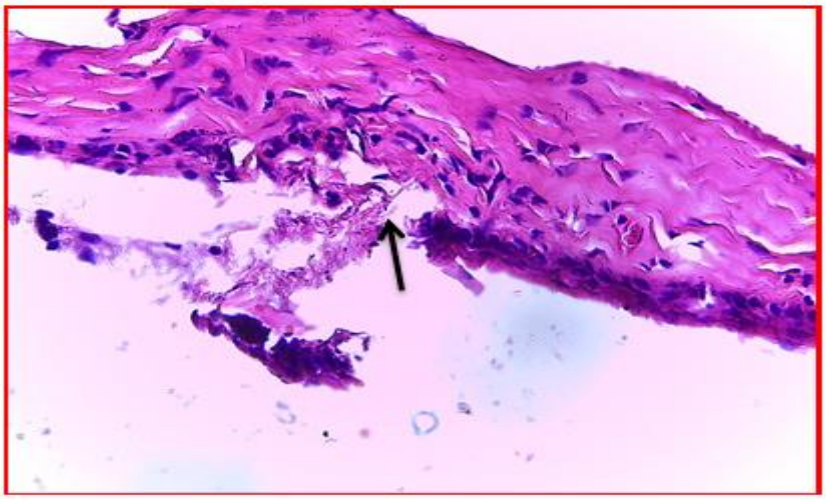

Figure 11) A section of the eye of infected mice treated with Cisplatin chemotherapy , showing sever necrosis of the corneal tissue, arrow.

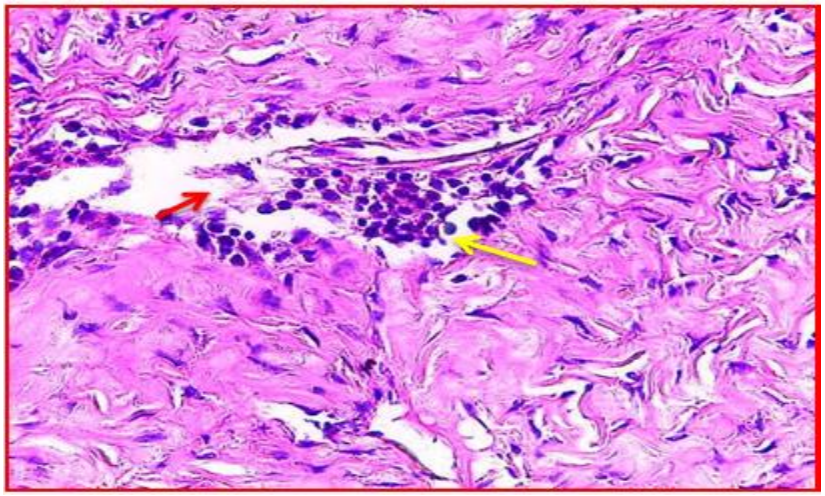

Figure 12) Inflammatory cells was observed in the deep areas of the cornea yellow arrow with sever tissue necrosis red arrow.

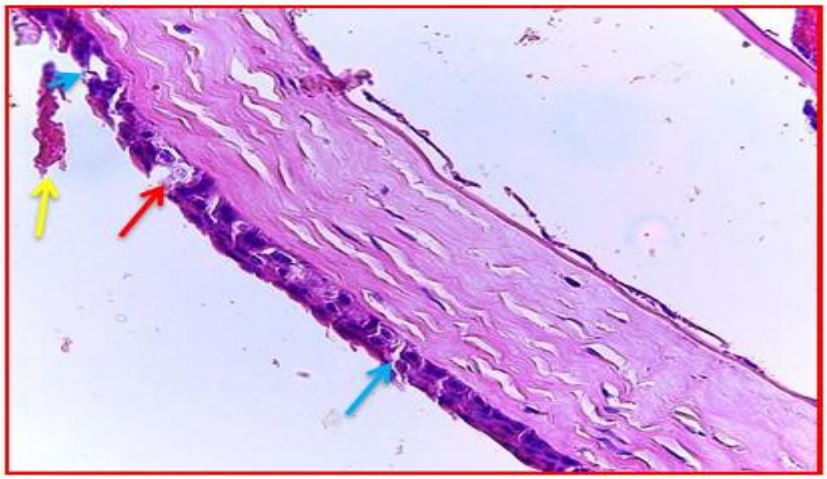

Figure 13) coronial tissue of infected mice treated with Chloramphenicol, the parasite was observed red arrow , with bleeding yellow arrow and tissue necrosis blue arrow .

DISCUSSION

The current study showed the ability of the *Acanthamoeba triangularis* to cause keratitis. This is

agree with many studies that have indicated that *Acanthamoeba spp*. cause eye inflammation. [12]
The use of DMSO had a fatal effect on the parasite, as no parasite found in the tissues of the eyes of infected mice treated with DMSO, but the compound caused scratching of the cornea, as this compound is a strong solvent that may have led to the extraction of fatty materials from the cells that make up the cornea. *Acanthamoeba* keratitis is difficult to treat and requires prolonged therapy despite the well-documented in vitro effectiveness of a variety of drugs this may be due to the cysts formed by the organism in response to hostile conditions[13].

DMSO, or dimethyl sulfoxide, is an organic solvent that triggers encystment and has been shown in some studies to be effective in preventing cysts from adhering to corneal epithelial cells. [14]suggested that DMSO be included in lens cleaning solutions due to its wide range of primary pharmacological actions, including membrane penetration, membrane transport, anti-inflammation, nerve blockade, bacteriostasis, and muscle relaxation.
The concentration that supports encystment was found to be lower in this study than it was in [14] research. Moreover, it was shown that at these doses, DMSO does not cause cytotoxicity in human corneal epithelial cells. However, given that past research has shown that cysts can stick to contact lenses, we believe that DMSO shouldn't be used in contact lens solutions.
[15]Therefore, even if the cysts' adhesion is weak, there's a chance that Acanthamoeba cysts might excyst and infiltrate the corneal epithelium, leading to an AK infection. Additionally, an epidemic in the USA was linked to a contact lens solution that included propylene glycol, a substance that caused Acanthamoeba trophozoites encystment, providing more evidence for this assertion. [16].

Many Studies show that DAMSO causes encystation of *Acanthamoeba*, and it is logical that the parasite encyst when it is exposed to not suitable condition, and the presence of the parasite in the corneal area may be unsuitable for the process of encystation because the parasite not have materials necessary for the encystation and this makes it effected by the compound which leads to death.
The use of Cisplatin cause severe effect on the eyes of experimental mice, as it did not cause the elimination of the parasite, in addition to causing more damage to the eyes. This may be due to the toxic effect of this drug, as this drug depends on stopping the process of cell division of cancer cells, Although cisplatin has anticancer properties through a variety of mechanisms, the most plausible one is the creation of DNA lesions by interaction with purine bases on DNA, which is followed by the activation of various signal transduction pathways and ultimately death.[17]

and it seems that this may explain the lack of death of the parasite because it has a slow rate of division, the parasite may persist for a long time without dividing, and this prevents its death, in addition to the fact that the drug may have poisoned the tissue of the eye, leading to necrosis. Its effect may be similar to the effect of chloramphenicol through inhibiting the process of protein synthesis and thus the death of the organism due to a defect in the functioning of its vital functions. Mocker found that the use of chloramphenicol caused a decrease in parasite numbers compared to control samples.

Conflict of Interest: The authors declare that they have no conflict of interest

Funding: No funding sources

Ethical approval: The study was approved by the University of Basrah College of Science Pathological analysis

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