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The use of *Matricaria chamomilla* aqueous extract as a treatment of mouse eyes experimentally infected with *Acanthamoeba castellani*

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Abstract

The protozoan Acanthamoeba can significantly damage vision. It enters eye through contaminated contact lenses or water and, immediately kills target cells. This study aimed to use Matricaria chamomilla aqueous extract as a treatment for Acanthamoeba castellani experimentally infected mouse eyes. Samples of Matricaria chamomilla plants were acquired from nurseries in the Basrah Province. Following the preparation of plant extracts, gas contact mass spectrometry (GC-MS) was used to identify the active components, showing that aqueous plant extract of Matricaria chamomilla included active substances like alkaloids, phenols, tannins, glycosides, and flavones. Corneal scrapings were collected from patients in Basrah Teaching Hospital. The isolated protozoan has been morphologically classified in the collected specimens to the genus level depending on cyst morphology, given the genus A. Castellani. Plant extracts from Matricaria chamomilla have been examined in vivo for their capacity to destroy Acanthamoeba cells. Acanthamoeba trophozoites (6 x 10^4 cells per ml) were inoculated into the eyes of healthy BALB/C mice (Mus musculus) and used for histological tests. The effects of Matricaria chamomilla aqueous plant extract on mice with healthy eyes showed that the epithelial layer healed, returning to its standard histological structure, and the protozoa went away.

Keywords: *Matricaria chamomilla*, plant extract, *Acanthamoeba*

Introduction

Acanthamoeba, a serious, sight-threatening, opportunistic protozoan that enters the eye through contaminated water or contact lenses is the leading cause of keratitis (AK). As soon as they enter the eye, infections cause them to kill target cells quickly. Contact lens wearers, who are associated with an increased risk of developing AK, account for up to 90% of cases [1].

Since there is no known cure or prognosis for AK, which primarily affects immunocompetent people, it is becoming more common. This has profound implications for eyesight [2].

According to [3], AK is a rare corneal infection that might endanger vision if not recognized and adequately treated. In the 1970s [4], was the first who recognized *Acanthamoeba* as an eye pathogen that causes chronic keratitis and is commonly

resistant to conventional antibiotic therapy.[5] Castellani discovered *Acanthamoeba* in 1930 [6].

One common free-living amoeba, *Acanthamoeba*, can be found in a variety of environments, such as the mucosa of nasopharynxes, skin lesions, contact lenses (CL), surgical tools, drainages, sediments, dialysis units, air, dirt, and dust [7].
[8] asserted that these creatures can also exist beyond the bodies of the infected hosts. According to [9], these organisms, can exist as active trophozoites or latent cysts and bloom in hot tubs, swimming pools, ponds, and contact lenses. According to [10], AK is the infection that most frequently affects CL users; there are 1 to 33 cases of AK infection per million CL wearers.

The well-known herb chamomile, *Matricaria chamomilla*, belongs to the Asteraceae family [11]. The German *M. chamomilla* has long been prized as a medicinal gem because of the breadth of its therapeutic uses [12]. It can adapt to various soil types, temperatures, and alkalinity. The "God of the Sun" gave this herb to the Egyptians as a priceless gift, which they cherished [13] .

This plant is regarded as a "star" herb because of its multiple aromatic and therapeutic qualities, according to [14]. These plants' essential oils (EOs) are crucial for aromatherapy, medicines, and natural food flavor [15]. German chamomile (*Matricaria chamomilla*) is the most widely used variation, with Roman chamomile being another well-known cultivar from the Asteraceae family [16].

Matricaria chamomilla is an annual or perennial plant indigenous to temperate regions of Asia, according to [17]. It is frequently planted worldwide, including in nations like western Xinjiang, China, Germany, Hungary, France, and Russia.

However, there are no published clinical trials to support the claims that *chamomile* has anti-inflammatory qualities, even though animal research has demonstrated this [18]; [19]; [20].

This study aims to determine how effective plant extracts from *Matricaria chamomilla* are against amoebas that cause AK, how often eye infections occur, and how they are treated.

Resources and techniques

Plant preparation

After being carefully cleaned, the plants were ground into a fine powder using a mechanical grinder (of the Modex variety) and kept in sterile containers until needed.

Extraction of *Matricaria chamomilla* in water

Plant extracts in aqueous form were developed, according to [21]. A glass flask containing 400 ml of hot, distilled water (500°C) and 20 g of dry plant powder was continuously turned for 12 h and 30 minutes. After that, The filtrate was put in Petri dishes and dried in the lab until it was needed at 40°C. A 45°C rotary evaporator (Orem Scientific Ltd., Switzerland) was then used to concentrate the paper.

Gas contact mass spectrometry (GC-MS):

For quality control reasons, labs of Nahran Omar-Basra Oil Company , the gas chromatography-mass Spectrometry (GC-MS) method was used to determine the chemicals produced from the extract of *Matricaria chamomilla*.

Aqueous extracts of *Matricaria chamomilla* qualitative reagents

Each *Matricaria chamomilla* flower was subjected to a series of quality checks using different chemical reagents made in a laboratory according to[22]: the phenol test, the flavonoids test, glycoside test, alkaloids test, Peach and Tracey test, the Saponins test, and the Tannins test .

Ethical approval

The ethical guidelines established by the Declaration of Helsinki were strictly observed when performing the study. Before a sample was taken, the patients' verbal and analytical consent was obtained.

Isolation of *Acanthamoeba*

73 corneal scrapings from the Basrah Teaching Hospital were allegedly taken from AK-infected people, including 27 people who wore contact lenses and 46 people who did not.

Samples were obtained from the lower conjunctiva and the eye using a sterile cotton swab rolled from one side to the other. Then, a light microscope was used to examine these samples. According to [23].

***Acanthamoeba* cultures**

1-PYGA: *Acanthamoeba* has been grown on PYGA culture. Many petri plates were sub-cultured to produce enough trophozoites and cysts to conduct the study.

2. non-nutrient agar: Was prepared by dissolving agar (15 g) (Sigma-Aldrich) in (1000 mL) of distilled water, then autoclaved at (121 °C) for (15)minutes, according to [24].

Staining and examination methods:

Prior to the investigation using the wet mount technique, two drops of the *Acanthamoeba* suspension were spread out on a glass slide, and the material was then covered off with a coverslip [25].

The final classification was based on the morphological level [26]. In addition to wet-mount staining techniques, transient stains like eosin and methylene blue were used.

Cell viability

Trypan Blue dye (0.4%) was used in a 1:1 ratio to test the vitality of *Acanthamoeba* cells [27]. *Acanthamoeba* cysts were measured with a hemocytometer. Cells were taken out of the growth medium after being centrifuged at 2000 rpm for five minutes and cleaned twice with sterile PBS saline. They were then counted using a Neubauer hemocytometer after being examined under a light microscope and injected into the mice's eyes.

Experiment animals :

158 BALB/C mice (*Mus musculus*) were used in the current investigation from both sex. They were all between three and four weeks old, weighing 20 and 30 g.

***Matricaria chamomilla* lethal dose (LD50):**

Matricaria chamomilla aqueous plant extracts were given to 5 groups of healthy BALB/C mice, each with six mice, at the following concentrations: 5, 2.5, 1, 25, 0.625, 0.3125, 0.15625, and 0.078125 mg/ml are the various concentrations. The experiment was repeated for each extract separately after 48 hours after counting the number of dead mice.

Animal inoculation

Trophozoites were produced from cultures and injected into the eyes of healthy BALB/C mice (6×10^4 cell per ml), according to [28]. After the final treatment, the employed animals were separated into three groups: one week, two weeks, and three weeks. Tissue culture preparation came next [29]. The data were examined using SPSS version 25, Duncan, LSD, ANOVA, and Duncan.

Matricaria chamomilla plant extract was tested for toxicity in mouse eyes without infection. *Matricaria chamomilla* aqueous extracts were added to the eyes of ten Balb/C-uninfected mice three times a day for three days at the least inhibitory concentration (0.1565 mg/ml) to test their toxicity on mouse corneas. Finally, animals were sacrificed, the findings were assessed, and sections were created.

Treating infected mouse eyes with *Matricaria chamomilla* aqueous plant extracts :

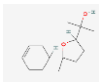
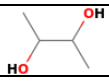
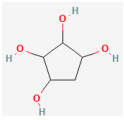
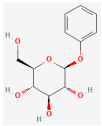
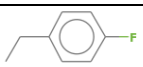
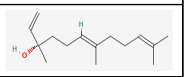
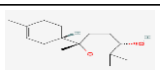
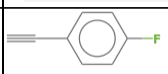
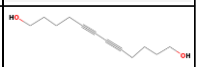
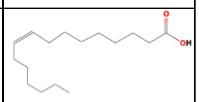
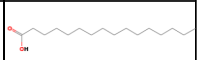
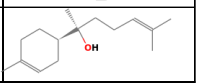
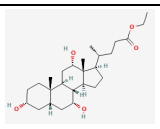
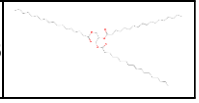
This extract therapy was started by using (2 groups of 10 infected balb/C mice) on the first day, and then the treatment was repeated for 3 days. The 1st group of infected mice consisted of 10 diseased balb/C mice, each receiving daily eye drops of 2 *Matricaria chamomilla* extract at 0.1565 mg/ml.

Results

The aqueous extract of *Matricaria chamomilla* contained 17 active compounds, including n-Hexadecanoic acid (4.77%), 2,3-butanediol (51.713%), bisabolol oxide B

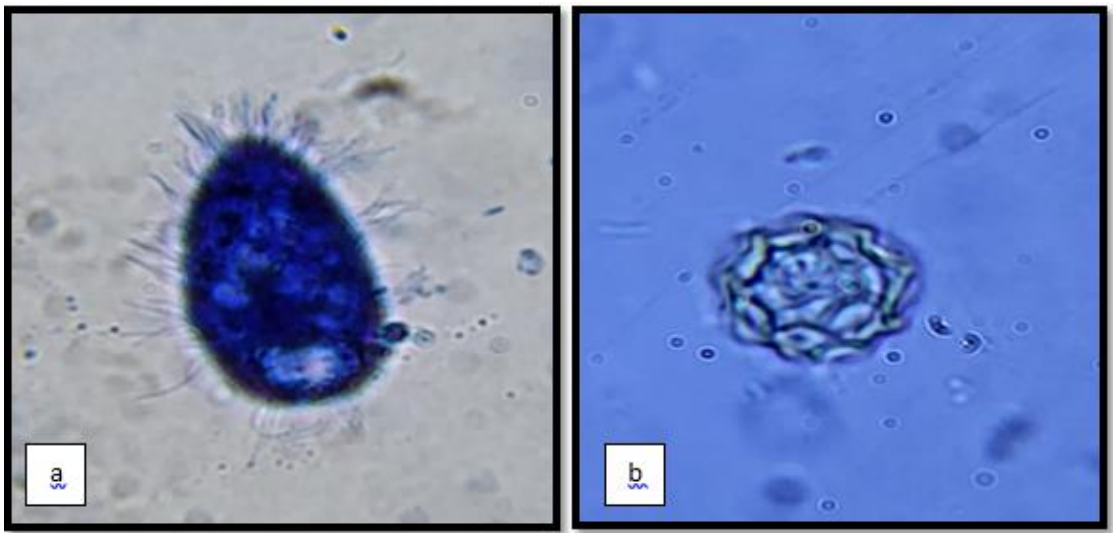
(5.0471%), unsaturated oleic acid (14.968%), 1,2,3-propane tri, 1 (E)-Nerolidol (4.456%), and finally 9-Octadecenoic acid.

Table 1 displays retention time (RT), percentages, areas, formulation, and chemical makeup of Matricaria chamomilla plant extract components

PK	Name	RT	Area	%	Formula	chemical compsition
1	Bisabolol oxide B	8.36	7185444	5.047157	C15H26O2	
2	2,3-Butanediol	8.883	205895813	51.71335	C4H10O2	
3	1,2,3,4-Cyclopentanetetrol, (1.alpha.,2.beta.,3.beta.,4.alpha.)-	9.267	2427231	0.60963	C5H10O4	
4	Phenyl-.beta.-D-glucoside	11.45	3083561	0.774476	C12H16O6	
5	p-Fluoroethylbenzene	13.021	3490224	0.876614	C8H9F	
6	(E)-Nerolidol	14.481	2172306	4.456023	C15H26O	
7	Bisabolol oxide A	15.101	11094632	2.786558	C6H6O2	
8	Benzene, 1-ethynyl-4-fluoro-	15.204	2466429	1.19475	C8H5F	
9	11-Dodecenoic acid, 10-hydroxy-, methyl ester	17.203	2419737	1.107202	C13H24O3	
10	5,7-Dodecadiyn-1,12-diol	18.442	4408305	2.667958	C12H18O2	
11	Palmitoleic acid	23.759	10622427	1.849004	C16H30O2	
12	n-Hexadecanoic	23.981	12399872	4.777813	C16H32O2	
13	Oleic Acid	25.706	2200518	14.96834	C18H34O2	
14	α-bisabolol	28.539	3372050	0.693966	C15H26O	
15	2,3-Dihydroxypropyl elaidate	30.117	2184152	1.371509	C21H40O4	
16	Ethyl iso-allocholate	36.268	7361779	0.696756	C26H44O5	
17	9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-	45.517	19022781	4.432793	C57H104O6	

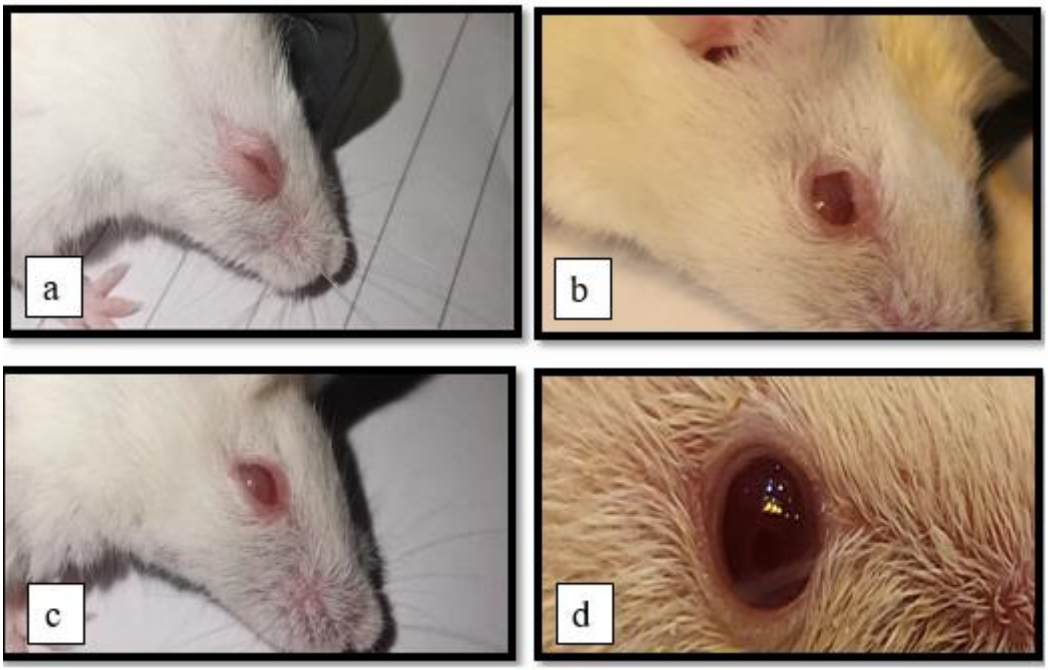
Most of the Matricaria chamomilla aqueous plant extract active components were alkaloids, phenols, tannins, glycosides, and flavones. Saponins and either were not present.

Trypan blue was used to find Acanthamoeba spp. in the clinical ocular sample. Cysts that were still alive did not stain, but cysts that were no longer alive did (Picture 1). Using the physical characteristics described on page 1967, we were able to confirm our findings and identify the species as "Acanthamoeba Castellani."



Picture (1) Trypan blue staining of Acanthamoeba castellani, 100X, a non-viable trophozoite (stained), and a viable cyst (non stained).

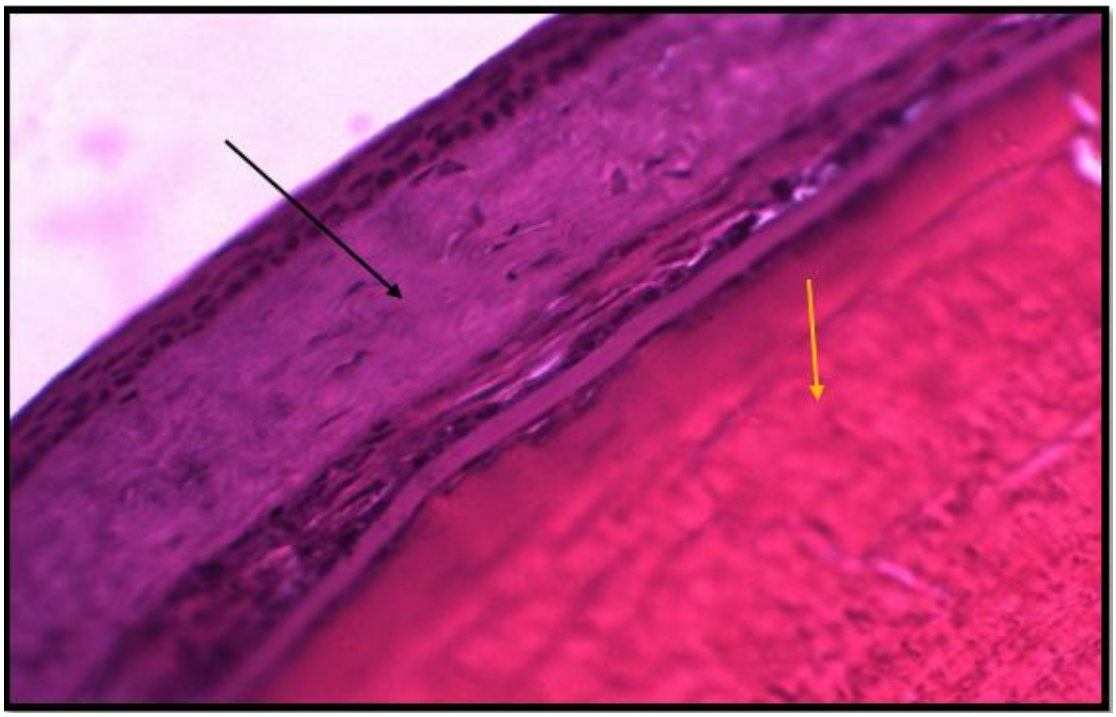
The AK signs in the mice's eyes were evident to the naked eye after the first day of induction (Picture 2). The cornea was destroyed, resulting in white circular ulcers with an enlarged center corneal white ring (Picture 2, b), which worsened the symptoms in the second week and eventually caused blindness (Picture 2, c). The symptoms of the first week were cloudy eyes, developing corneal infections on days two and three, infection in the conjunctiva, and edema. The control groups, in comparison, showed no signs of AK (Picture 2, d).



Picture (2) Weeks 1–3 of AK symptoms in an infected eye: A mouse eye with edema in the first week of infection; B a mouse eye with an inflamed cornea and a white ring; C a mouse eye with melting of the cornea; D a mouse eye that is normal (control).

Numerous histopathological variations have been observed in the first week after infection, such as destruction of Bowman's layer with ulceration in the epithelial layer, inflammatory implies (composed of neutrophils) in the stroma, stroma

vascularization (in the mid and deep peripheral part of the stroma), trophozoites, and cyst stages in the epithelial layer and stroma (Picture 3).In addition, several clusters of inflammatory cell infiltrates were found in the deeper stroma and the area around Descemet's membrane. Additionally, acinar fibrosis, lacrimal gland metaplasia, and capillary congestion were found. In the second week of infection, it was also discovered that it was causing the lacrimal cells to vanish and some of the acinar lacrimal gland's cells to degenerate. The third week of infection was also observed to cause cyst stage and trophozoite stage lacrimal gland breakdown in the study. Third-week histological abnormalities in the skin of the eyelids include dermal degradation, sebaceous gland fibrosis, the disappearance of the hair bulb, the existence of trophozoite and cyst stages, and significant infiltration of immune cells and RBCs.

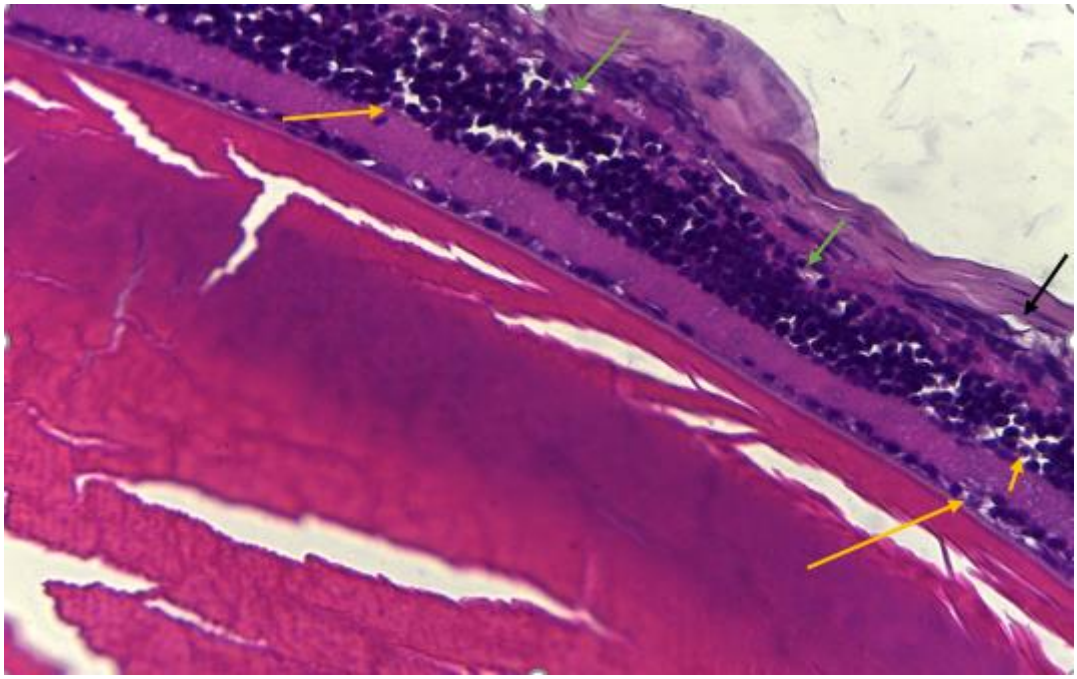


Picture (4) Normal layer of corneal epithelium (black arrow), normal lens (yellow arrow), and normal eye structure (control) as seen at 40x in H&E.

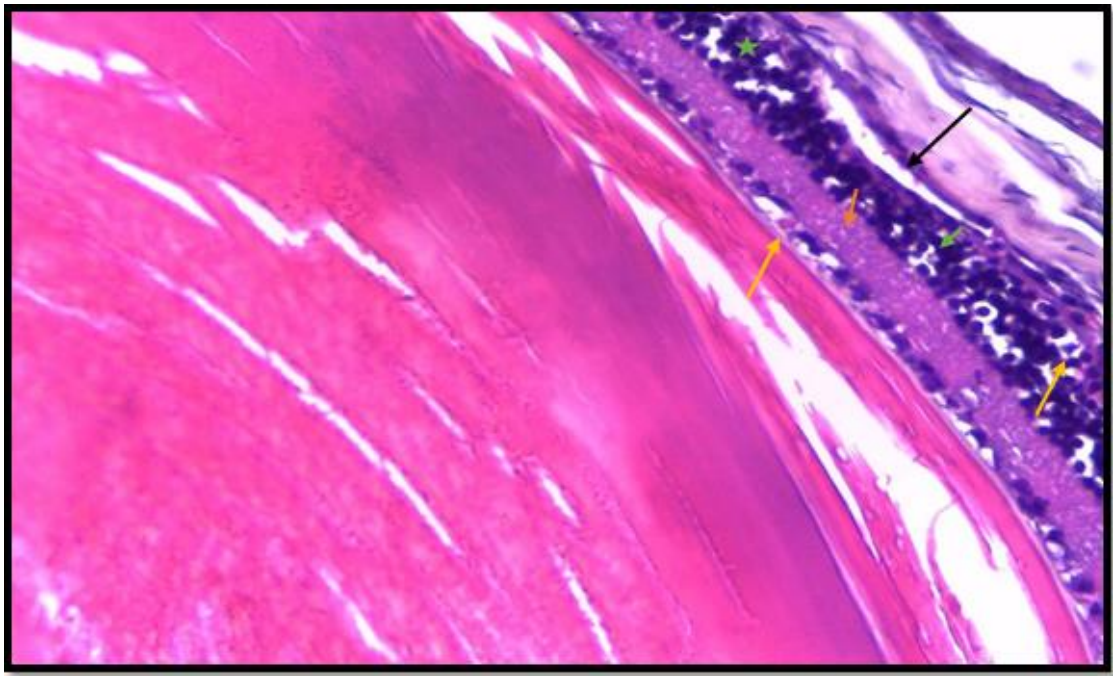
Matricaria chamomilla aqueous plant extract's toxicological impact on mice's eyes without infection:

According to the study, when tested on mice's eyes that were not diseased, an aqueous plant extract from Matricaria chamomilla showed no toxicological effects. The eyes' cornea, stroma, and epithelium remained normal, and there were no histological alterations, as seen in Picture 12.

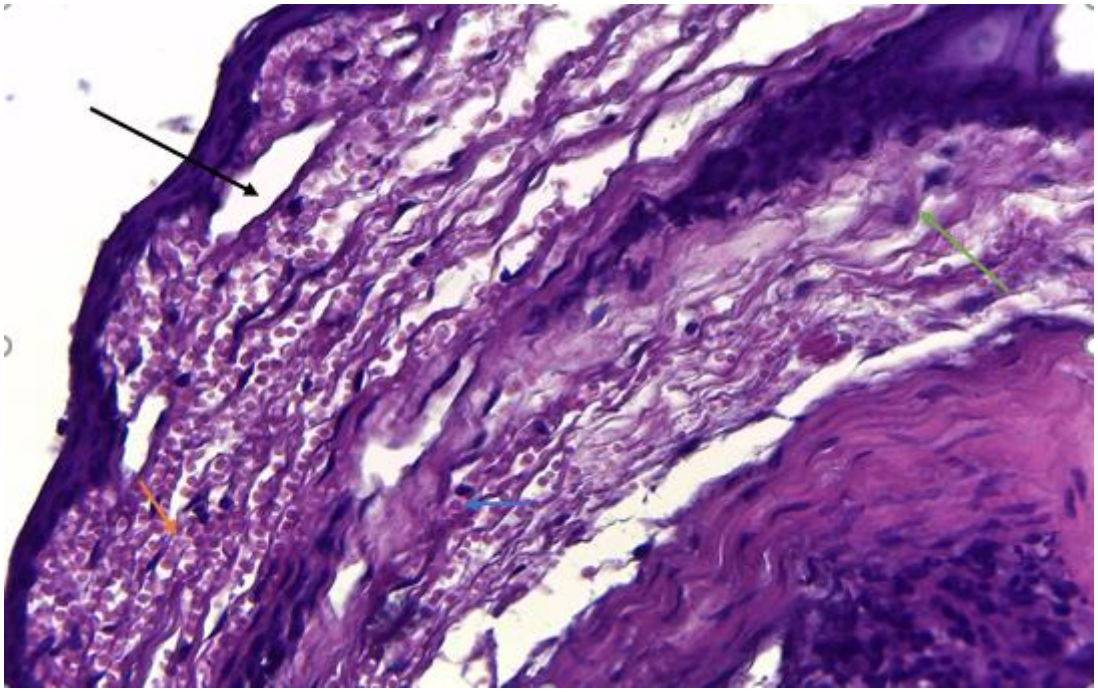
Acanthamoeba extract from *Matricaria chamomilla* is used to treat the eyes of AK-infected mice. As depicted in Picture 15, an extract from *Matricaria chamomilla* was used to cure infected mouse eyes only when treating was in the early stages ,the edema can go away and the eye return to its normal state; late stages are incurable and can result in blindness . Additionally, after treatment with *Matricaria chamomilla* aqueous plant extract, the epithelial layer was healed, both *Acanthamoeba* stages vanished, and the mouse eyes recovered their standard histological structure.



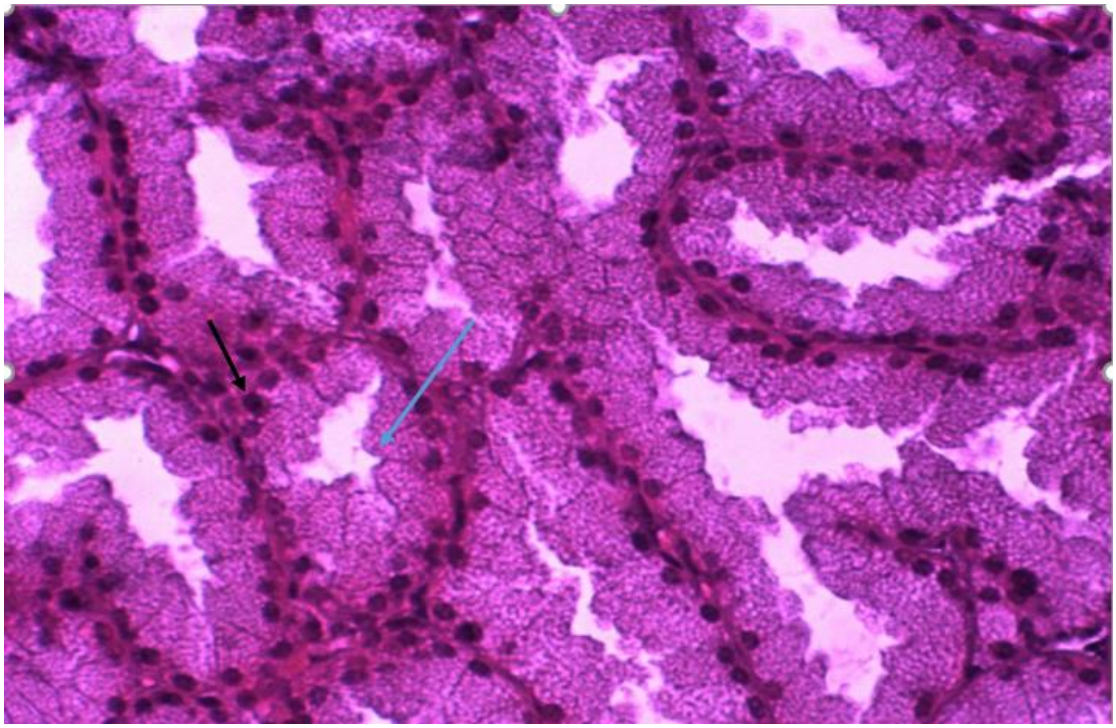
Picture (5) Infected mouse eyes (first week) showed trophozoite stages (yellow arrows), inflammatory cells (green arrows), and damage in the corneal epithelial layer (black arrows).



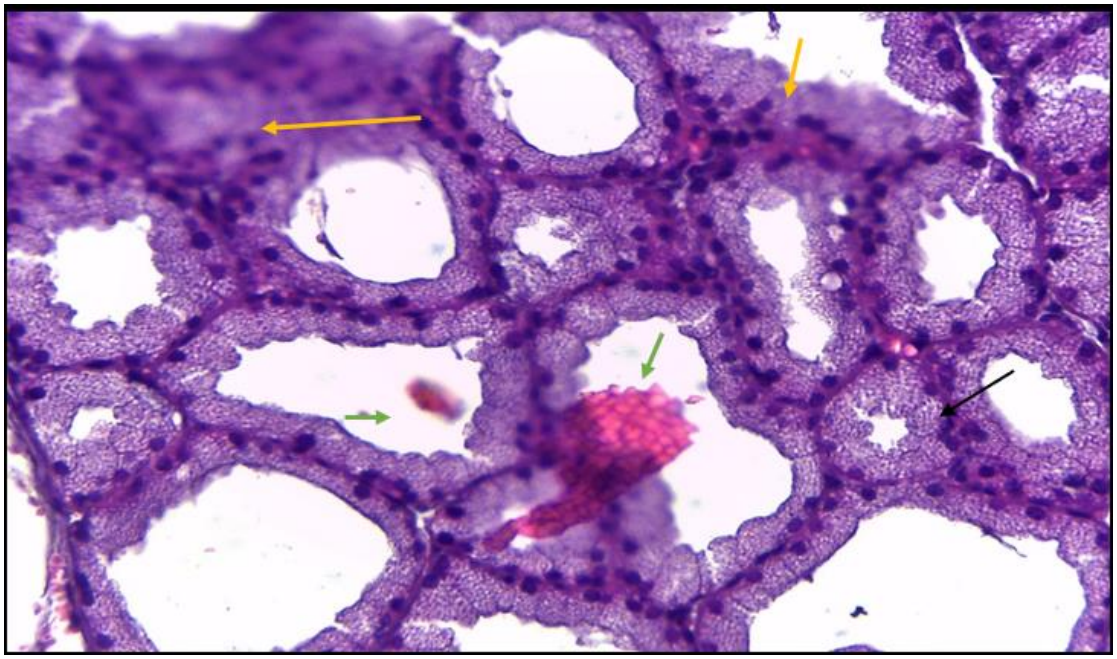
Picture (6) Infected mouse eye, second week, displaying trophozoite stage (yellow arrow), cyst stage (green arrow), RBC presence (orange arrow), many inflammatory cells (stick), and corneal epithelial layer degradation (black arrow).



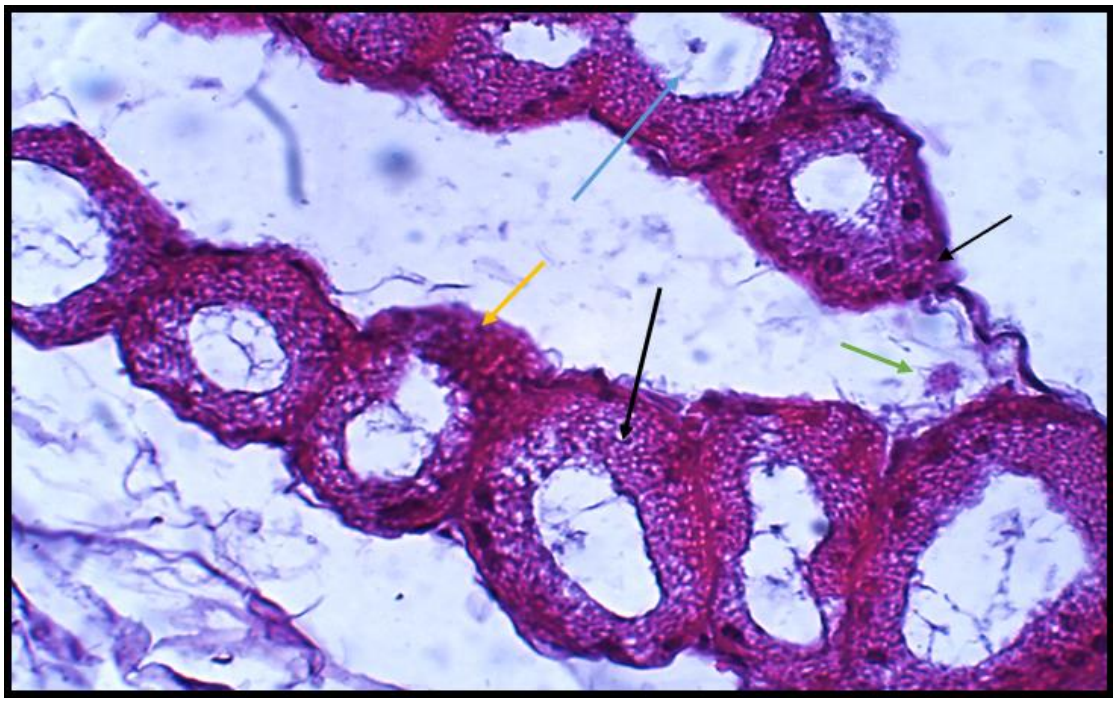
Picture (7) The corneal epithelial layer in the infected mouse eye in the third week showed severe damage and disintegration (black arrow), the occurrence of red blood cells , inflammatory cells (orange arrow), trophozoites (green arrow), and cysts (blue arrow) (40x, H&E).



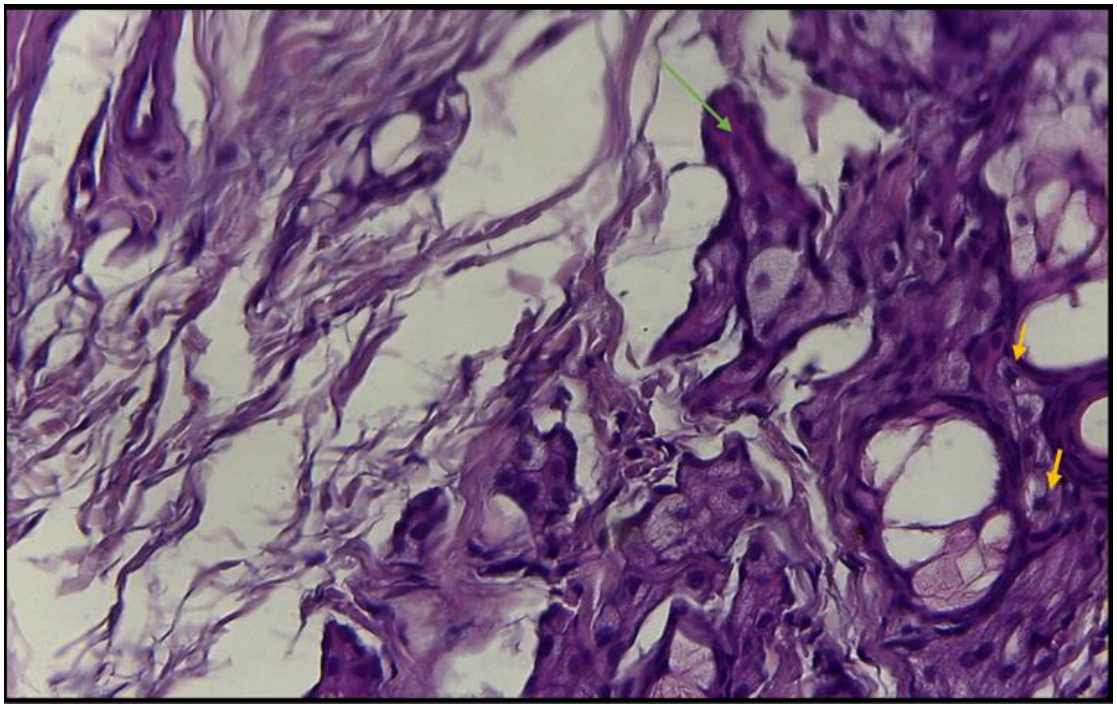
Picture (8) Healthy (control) At a magnification of 40x in H&E, the lacrimal gland exhibits healthy characteristics (blue arrow) of large nucleated cells (black arrow).



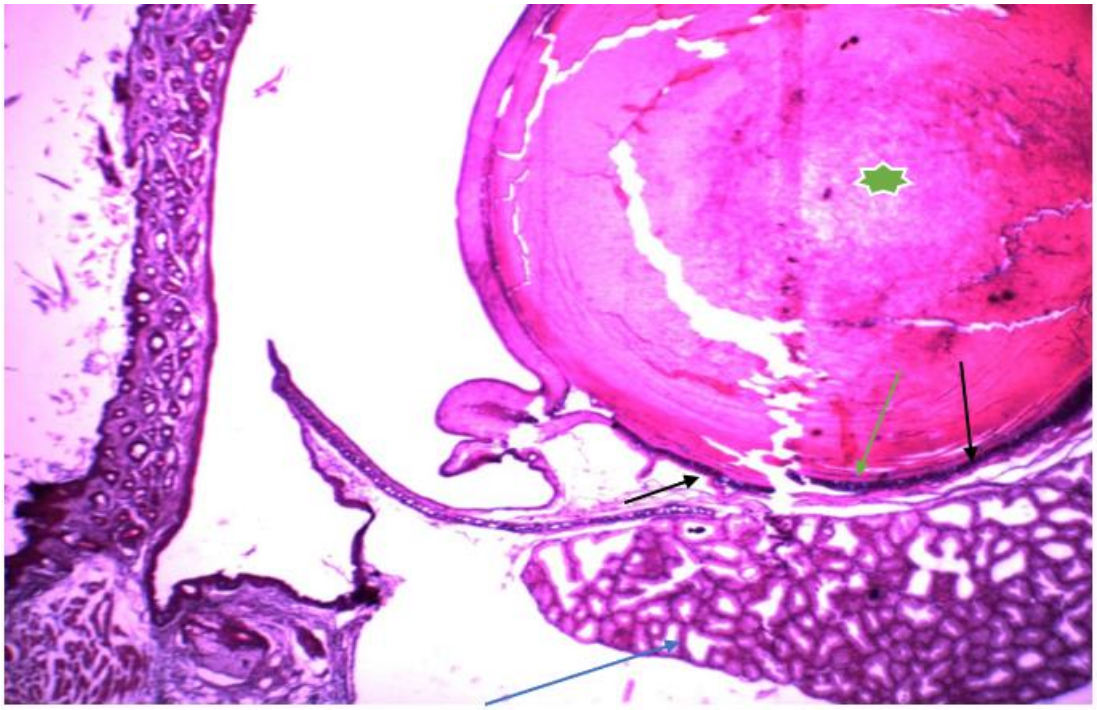
Picture (9) Acinar fibrosis (yellow arrow), metaplasia (black arrow), and capillary congestion in the lacrimal gland that is visible in the first week of mouse eye infection.



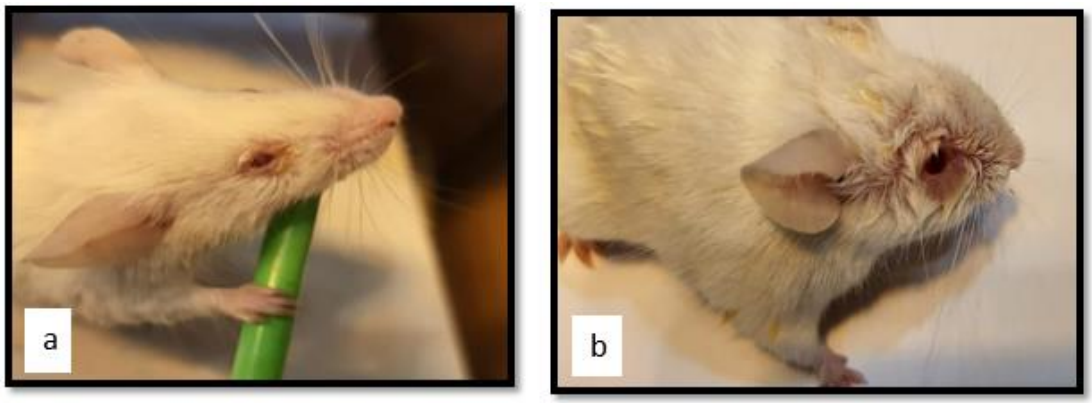
Picture (10) Second week of infection shows the trophozoite and cyst stages (green and blue arrows, respectively), as well as the removal of the nucleus of the lacrimal gland (black arrows) and cell degeneration in a few of its cells (yellow arrows), 40x, H&E.



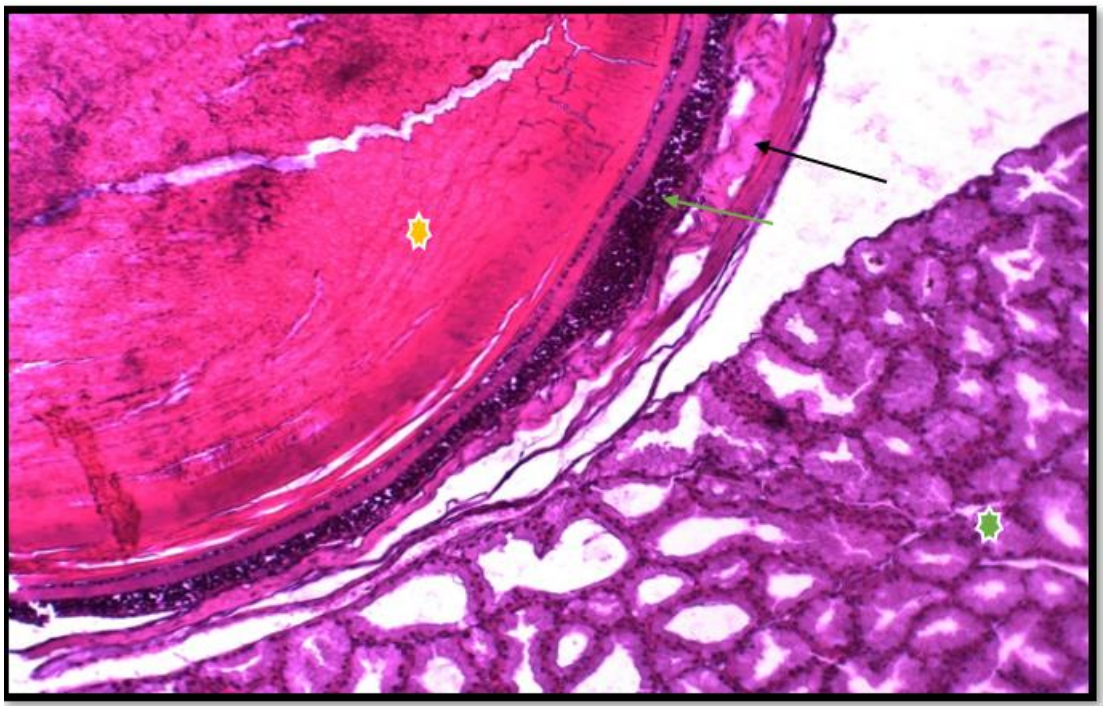
Picture (11) Third week of infection displaying trophozoite stage (yellow arrows) and breakup of lacrimal gland (green arrows) at 40x, H&E.



Picture (12) Normal epithelium layer (black arrow) and stroma (green arrow) observable in an uninfected mouse eye. After employing Matricaria chamomilla aqueous plant extract H&E stain 40X, the cornea (stick) and lacrimal gland (blue arrow) are seen.



Picture (13) a- Early infection stage of mice eye with Acanthamoeba castellani b-infected eye following therapy with a Matricaria chamomilla plant extract.



Picture (14) infected mice eye After treating with Matricaria chamomilla aqueous plant extract H&E stain 40X, healthy epithelial layer (black arrow), a healthy stroma, cornea, and lacrimal gland (yellow and green sticks), all of which are clear of Acanthamoeba.

Discussion

Few compounds that are effective against parasitic infections are used as antiseptics or as treatment due to factors like the inability of these chemicals to cross the (blood-brain barrier)and the robustness of the cyst stage and its resistance to severe circumstances [11].

However, chemists have created several substances that could be utilized to treat a number of endoparasites. Furthermore, the discovery of plant extracts and their application for AK therapies, as employed with many other parasites, have not received much attention in recent investigations. Due to the rarity of illnesses brought on by free-living amoebae and the lack of awareness among physicians, the discovery of antiparasitic disease drugs is also not adequately rewarded [30].

The popular aromatic medicinal herb *Matricaria chamomilla* is mostly utilized for its therapeutic effects. The most popular items are dried flowers, which have a variety of medical characteristics, including analgesic, anti-inflammatory, antibacterial, anti-spasmodic, and sedative effects [31]. The current study's findings indicate that antibacterial properties can be found in substances such as chamazulene, bisabolol umbelliferon, and cyclic ethers. This is comparable to [32] study; it was found that the components in the extracted chamomile flower were the same. The cytotoxic effects of plant extracts on mice and their anti-amoebic abilities against *Acanthamoeba castellani* were assessed in vivo.

The results of the present study show that these compounds considerably retard the proliferation of pathogenic *Acanthamoeba*[25]. There aren't many studies on how well *Matricaria chamomilla* plant extracts work against *Acanthamoeba*[13]. One of them has been discovered to provide a variety. They may be effective against infections caused by free-living amoebae because of their variety of therapeutic benefits, which include anti-inflammatory, antiviral, antibacterial, antidiabetic, and antiprotozoal properties [33].

Because the cyst stage of *Acanthamoeba spp*. has the strongest layers of cyst walls, it is widely known to be resistant to a number of environmental stressors, such as extreme weather [34]; [35]. According to recent research, *M. chamomilla* plant extracts inhibited *Acanthamoeba castellani* trophozoites and cyst forms, and aqueous extracts demonstrated potent anti-Acanthamoebic activities.

Thorough destruction and inhibition have been shown to occur when using *Matricaria chamomilla* aqueous plant extracts.

According to [36] certain plant species contain bioactive chemicals that have been shown to have strong amoebic effects on *Acanthamoeba castellani*, preventing its encystation and excitation. Oleic acid, which has an antimicrobial activity and can induce cell death, is present in medicinal plant extract, This is similar to a study by [37], which proposed that oleic acid significantly obstructs the growth of the trophozoites because of its critical role in causing the destruction of the structure in the membrane's lipid. Additionally, the results imply that *Acanthamoeba castellani* proliferation may be inhibited by oleic acid therapy by inducing apoptosis and trophozoite autophagy. The current study's findings imply that the high flavonoid concentration of these plant extracts contributes to the destruction of *Acanthamoeba spp*.'s wall stability and inhibits both stages of the organism. This is comparable to a study by [38] that showed flavonoids can stop microbe growth by depleting the membrane and preventing the creation of DNA, RNA, and even proteins, according to the estimation of the LD50 of used aqueous plant extracts, that appears with no lethal properties on mice (no proportion of mice died across all groups).

The dose utilized in the current investigation ($6*10^4$) is lower than that in prior studies, and it has been demonstrated to cause a severe infection in the eyes, demonstrating the virulence of the isolated amoeba that caused AK to infect mice's eyes and cause a quickly progressive sickness. Involves a number of pathological changes, including trophozoites and cysts in the

corneal stroma, stromal necrosis, corneal epithelial cells necrosis, extracellular matrix incursion, and decrease of the keratocyte, and finally, inflammation may occur. All of these changes were similar to those seen by De Lacerda et al. when studying *Acanthamoeba* keratitis in experimental animals.

The results of the current investigation showed that *Matricaria chamomilla* aqueous plant extracts applied to the eyes appeared non-lethal in the quantities employed and showed no histological alterations; hence, their in vivo activity was tested in this study.

According to the study's findings, *Matricaria chamomilla* aqueous extracts at a concentration of 0.625 mg/ml are extremely effective at killing the amoeba stages. Additionally, when applied in the previously mentioned concentrations, this extract was very effective in curing infected mice's eyes, and if applied in the early stages of infection, it could stop the progression to brain illness.

Conclusion and recommendations

According to the current study, the infection with *Acanthamoeba Castellani* has been increased 48% more than recent investigations. The results show that aqueous plant extracts with a concentration 0.625 mg/ml of *Matricaria chamomilla* was successful for treating the infections in mouse eyes.

This study is the first in the world that used these plant extracts against *Acanthamoeba castellani protozoan* . Early finding and treatment of the protozoan in the first week is very important to stop the development of brain infection which leads to lethality. Finally, it may be argued that it is crucial to conduct additional research on this parasite.

Conflict of Interest:

The authors declare no conflict of interest.

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استخدام مستخلص *البابونج المائي* كعلاج لعيون الفئران المصابة تجريبيا بطفيلي الـ *Acanthamoeba castellani*

الأكانثاميبا من الاوالي التي ممكن ان تحطم النظر. يدخل العين من خلال العدسات اللاصقة الملوثة أو الماء ويقتل الخلايا المستهدفة على الفور. تهدف هذه الدراسة إلى استخدام المستخلص المائي *Matricaria chamomilla* كعلاج لعيون الفأر المصابة مختبريا بـ *Acanthamoeba castellani* . تم الحصول على عينات من نباتات *البابونج* من المشاتل في محافظة البصرة. بعد تحضير المستخلصات النباتية ، استخدم مطياف الكتلة الملامسة للغاز (GC-MS) لتحديد المكونات النشطة ، والذي اظهر ان المستخلص النباتي المائي من نبات البابونج يحتوي على مواد فعالة مثل القلويدات والفينولات والعفص والجليكوسيدات والفلافونات. جمعت عينات من القرنية من المرضى في مستشفى البصرة التعليمي. صنف الأوالي المعزولة مظهريا في العينات التي تم جمعها إلى مستوى الجنس اعتمادا على شكل الطور المتكيس ، على انها من جنس *A. Castellani*. فحصت المستخلصات النباتية للبابونج في الجسم الحي لمعرفة قدرتها على تحطيم خلايا *Acanthamoeba*. حقن الطور الخضري للـ *Acanthamoeba* ($10^4 \times 6$ خلية|مل) في عيون الفئران السليمة BALB / C) ثم استخدمت في الاختبارات النسيجية. أظهرت تأثير المستخلص المائي لنبات *Mus musculus* من سلالة البابونج العيون السليمة للفئران أن الطبقة الظهارية التأمت ، وعادت إلى بنيتها النسيجية القياسية ، كذلك اختفت الأوالي.