parasitologia

Review

Amebicides against *Acanthamoeba castellanii*: The Impact of Organism Models Used in Amebicide Assays

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Abstract: *Acanthamoeba castellanii* is a free-living amoeba capable of causing keratitis in humans, with most cases related to contact lens wearers and surgical procedures. In addition, *A. castellanii* may cause pneumonia, granulomatous encephalitis, and skin lesions in immunocompromised individuals. Considering the lack of adequate treatment for acanthamoebiasis, the aim of this review is to assess relevant original articles that covered the current arsenal of drugs and models of organisms used in the field of experimental *A. castellanii* infection that have been published within the last 5 years (2018–2023) in journals indexed by the following databases: Electronic Library Online (SciELO), PubMed, Medical Literature Analysis and Retrieval System Online (Medline), Latin American and Caribbean Literature in Health Sciences (Lilacs), Google Academic, and Capes Periodical Portal. Thirty articles were selected, and the main findings showed that the available therapeutics for acanthamoebiasis are still limited and nonspecific, and no innovations have occurred in the last few years. In terms of novel chemotherapeutic advances, the last findings have focused on the activity of natural products (plant-based extracts), nanoemulsions, coated particles, and photodynamic association against *A. castellanii*, without advancing from the bench to bedside perspective. The choice of a non-representative model system for acanthamoebiasis, as well as the limitations of studies in vivo, impairs the advancement of toxicity analyses. Efforts should be made to expand the model systems used, standardize tests for evaluating anti-*A. castellanii* drug candidates, and increase and support research groups focusing on the biology of *A. castellanii* and the pharmacology of acanthamoebiasis.

Keywords: acanthamoebiasis; antiparasitic agents; drug therapy; pharmacology

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1. Introduction

A. castellanii is a free-living protozoan with varied sizes (20–30 µm) (Figure 1), which, in its trophozoite form, often occurs in water-related environments and reproduces through mitosis; it may also be present in environments with the presence of biofilm of microorganisms. However, in its cystic form (13–23 µm), *A. castellanii* can be widespread in all environments because the cyst is resistant [1,2] (Figure 1). In general, when the amoeba identifies adverse environmental conditions, it changes its form from trophozoites to cysts to protect the genetic material, which can be transmitted through the air and travel through systems, including long-distance ventilation ducts, high winds, and dust storms [2,3].

Although considered a rare disease, cases of keratitis caused by *A. castellanii* have increased due to the popularization of contact lenses use, which, when poorly sanitized with solutions contaminated with amoeba cysts or trophozoites, lead to chronic corneal infection in immunocompetent individuals [3–6]. Keratitis caused by amoeba is an inflammation of the corneal tissue that leaves the tissue transparent or opaque and causes eye redness, pain,

blurred or diminished vision, and occasional loss of vision due to parasite penetration and cytotoxic and phagocytic amoeba activity [1,5–9].

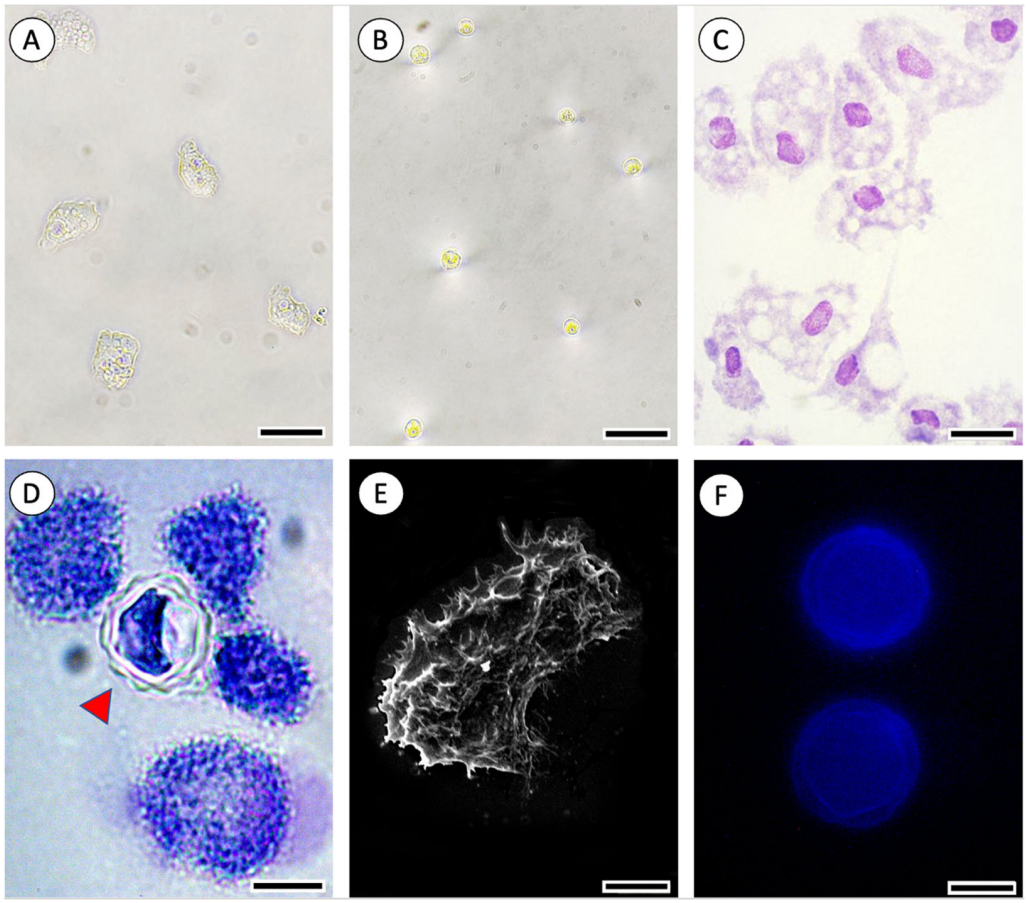


Figure 1. Cysts and trophozoites of *A. castellanii*. (**A**) Trophozoites of *A. castellanii* under an inverted microscope. Scale bar = 20 µm. (**B**) Cyst induced in Tris buffer solution under an inverted microscope. Scale bar = 40 µm. (**C**) Trophozoites stained in Giemsa. Scale bar = 10 µm. (**D**) A cyst (red arrow) induced by the deprivation of a rich medium stained in Giemsa. Scale bar = 10 µm. (**E**) Trophozoites in a scanning electron microscope. Scale bar = 7 µm. (**F**) Cyst autofluorescent in fluorescence microscopy. Scale bar = 10 µm.

The prevalence of amoeba keratitis has increased due to the lack of specific antiseptic methods for amoeba decontamination during ocular surgical procedures and the routine use of contact lenses [10]. A limited number of studies have focused on acanthamoebiasis epidemiological surveillance; data show that the incidence between the 1990s and 2019 in the United States was approximately 105 cases, and in the UK, there were 243 cases and in Brazil, there were 185 cases per million [3,10–12].

In immunocompromised patients, *A. castellanii* causes pneumonia, granulomatous amoebic encephalitis, and skin ulcers. In such cases of skin ulcers, the patient must have presented a previous wound that allowed contact with trophozoites or cysts [7,13,14]. Pneumonia develops when an individual breathes dust particles containing amoeba cysts that decystize in the lungs and initiate phagocytosis of surrounding cells, causing damage to the lungs and ultimately leading to cough, thoracic pain, and shortness of breath. These symptoms often lead to confusion with bacterial pneumonia [7,14,15].

Amoeba can reach other human systems, especially the nervous system, through the skin and ocular lesions, as well as the oral and respiratory routes. In this case, there is a risk that patients will develop granulomatous encephalitis, which is characterized by confusion,

low-grade fever, headache, blurred vision, mood swings, cognitive problems, paralysis, and seizures. Patients may die within one week without treatment [9,14,16–18].

Therefore, *A. castellanii* is a protozoan of medical importance, with relevant morbidity and mortality potential, resulting in a complex scenario for which therapy is far from effective and specific [7]. Several factors complicate the treatment of the clinical forms of acanthamoebiasis. First, these are unusual manifestations that are not a priority for large-scale clinical studies. In addition, the virulence of free-living amoeba is not necessarily comparable to laboratory strains, making it difficult to trace the relationships between in vitro, in vivo, and bench-to-bedside findings [7].

Owing to the medical importance of accidental and/or opportunistic infections of *A. castellanii* in humans and the lack of specific therapies, it is essential to advance the development of new and more effective therapies [3,7]. There have been recent reviews dealing with acanthamoebiasis treatment, but these addressed specific topics such as nanoparticles for keratitis, delivery systems for keratitis, and synthetic compounds and discussed key research areas [1,3,6,7]. Therefore, the aim of this integrative review was to collect studies carried out in the last five years (2018 to 2023) that describe the utilization of amoebicidal drugs against acanthamoebiasis in clinical practice and comprehend the current research that has been conducted to identify candidates with anti-*A. castellanii* activity. We list the main drugs used in the treatment of acanthamoebiasis in the clinical practice, their mechanisms of action, and the main associations of drugs in two detailed tables (Tables 1 and 2) and also the general characteristics (IC50, isolate/stage, assays, cell model, and mechanism of action and observations) of the main studies on amoebicidal drugs in past five years (2018–2023) (Tables 3 and 4). We also propose an analysis of model organisms and model systems used in the study of drugs loved against *A. castellanii*.

Table 1. Main associations of drugs used in the treatment of acanthamoebiasis clinical manifestations.

Clinical Manifestations	Association Drugs for the Treatment
Keratitis	Polyhexamethylbiguanide + Propamidine Isethionate + Hexamidine + Antibiotics (Gatifloxacin or Ciprofloxacin) + Antifungal (Clotrimazole or Miconazole) + Miltefosine
	Chlorhexidine + Propamidine Isethionate + Hexamidine + Antibiotics (Gatifloxacin or Ciprofloxacin) + Antifungal (Clotrimazole or Miconazole) + Miltefosine
Skin ulcers	Miltefosine + Pentamidine + Antifungal (Fluconazole or Itraconazole) + Antibiotics (Azithromycin or Clarithromycin)
	Amphotericin B + Miltefosine + Sulfamethoxazole + Trimethoprim + Sulfadiazine + Flucytosine
	Metronidazole + Paromomycin
	Mebendazole + Ivermectin + Praziquantel
	Tinidazole or Secnidazole + Nitazoxanide
Granulomatous encephalitis	Miltefosine + Pentamidine + Antifungal (Fluconazole or Itraconazole) + Antibiotics (Azithromycin or Clarithromycin)
	Amphotericin B + Miltefosine + Sulfamethoxazole + Trimethoprim + Sulfadiazine + Flucytosine
Pneumonia	Antiparasitic (Secnidazole or Tinidazole or Metronidazole + Paramomycin + Nitazoxanide) + Antifungal (Fluconazole or Itraconazole) + Antibiotics (Azithromycin or Moxifloxacin or Levofloxacin or Amoxicillin) + Ceftriaxone

Table 2. Main drugs used in the treatment of acanthamoebiasis in clinic practice and their mechanisms of action.

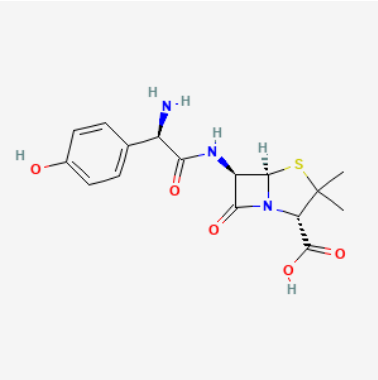
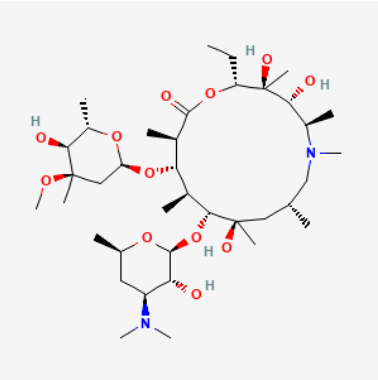
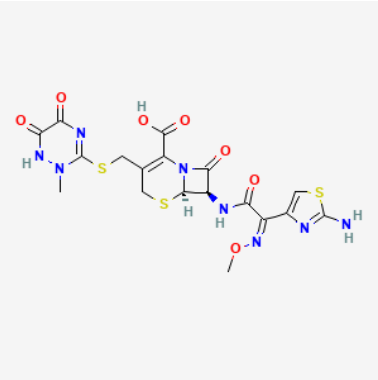
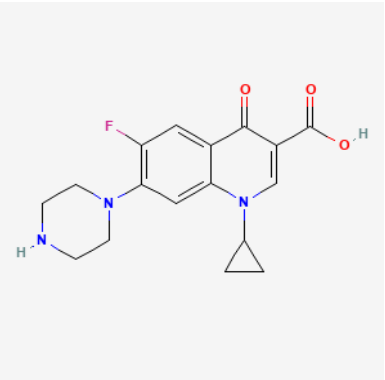
Drug of Choice	Mechanism of Action Known for Microrganisms	Descriptions and Effects on *A. castellanii*	Chemical Structure	Reference
		Antibiotic		
Amoxicillin	Inhibits bacterial cell wall synthesis of *Proteus mirabilis*, *Streptococcus pyogenes*, and *Neisseria gonorrhoeae*	Not available		[19]
Azithromycin	Binds to the 50S ribosomal subunit, inhibiting essential protein synthesis of *Legionella pneumophila*, *Chlamydia* ssp., and *Fusobacterium* ssp.	Effective adjuvant to standard anti-*A. castellanii* chemotherapy		[20]
Ceftriaxone	Inhibits bacterial cell wall synthesis of *Escherichia coli*, *Klebsiella pneumoniae*, and *Streptococcus pneumoniae*	Not available		[21]
Ciprofloxacin	Inhibits DNA gyrase and DNA topoisomerase, preventing transcription and replication of the genetic material of *Staphylococcus aureus*, *Salmonella* spp., and *Campylobacter jejuni*	Not available		[22]

Table 2. *Cont.*

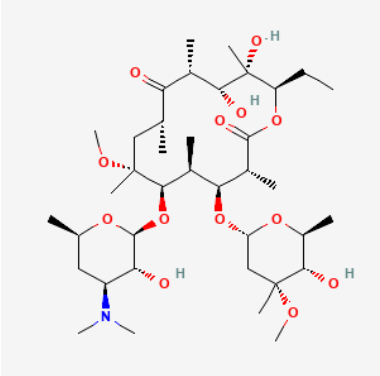
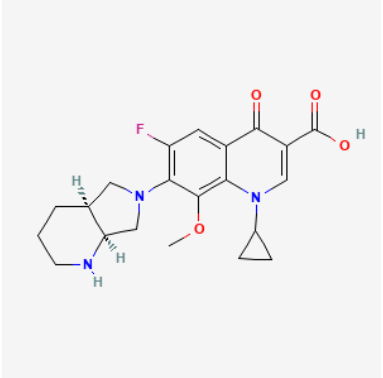
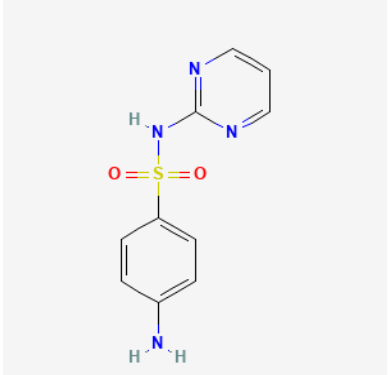
Drug of Choice	Mechanism of Action Known for Microrganisms	Descriptions and Effects on *A. castellanii*	Chemical Structure	Reference
Clarithromycin	Binds to the 50S ribosomal subunit, inhibiting the protein synthesis of *S. pneumoniae*, *Haemophilus influenzae*, and *Helicobacter pylori*	Not available		[23]
Gatifloxacin	Inhibits DNA gyrase and DNA topoisomerase, preventing the transcription and replication of *S. pneumoniae* and *Moraxella catarrhalis*	Cysticidal capacity		[24]
Moxifloxacin	Acts on topoisomerase enzymes, preventing DNA replication and repair of *Enterococcus faecalis*, *E. coli*, and *K. pneumoniae*	Low effectiveness against granulomatous amoebic encephalitis		[25]
Sulfadiazine	Inhibits the folic acid synthesis and metabolism of *S. pneumoniae*, *S. aureus*, and *E. coli*	Low effectiveness against cysts		[26]

Table 2. *Cont.*

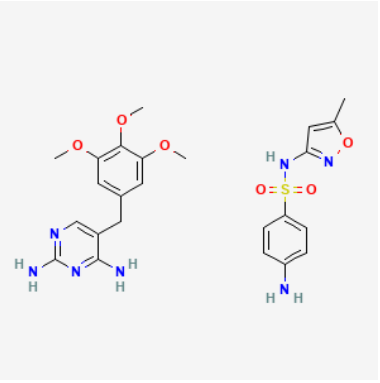
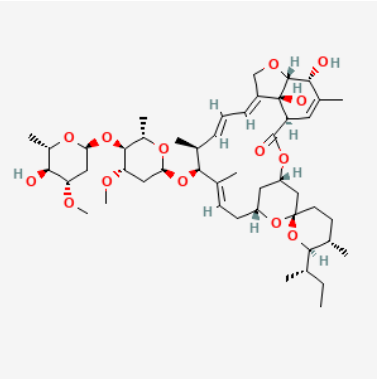
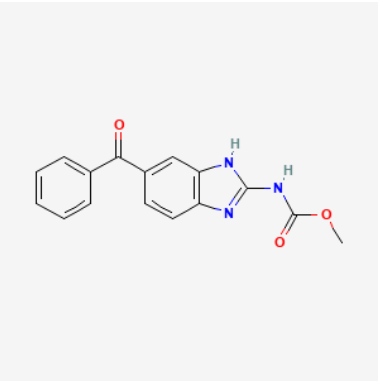
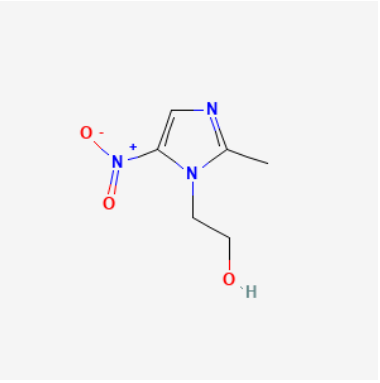
Drug of Choice	Mechanism of Action Known for Microrganisms	Descriptions and Effects on *A. castellanii*	Chemical Structure	Reference
Sulfamethoxazole-trimethoprim	Inhibits enzymes that are related to the folate biosynthesis of *K. pneumoniae*, *Proteus* spp., and *Salmonella* spp.	Cytotoxic activities against trophozoites and cysts		[27]
Antiparasitic				
Ivermectin	Induces tonic paralysis of the musculature of parasites through the interference with the chloride channels of *Sarcoptes scabiei*, *Pediculus humanus capitis*, and *Ascaris lumbricoides*	Not available		[28]
Mebendazole	Interrupts cell replication, inhibiting microtubule synthesis and the glucose uptake cascade of *Enterobius vermicularis*, *A. lumbricoides*, and *Ancylostoma duodenale*	Not available		[29]
Metronidazole	Inhibits DNA synthesis of *Trichomonas vaginalis*, *E. histolytica*, and *Giardia lamblia*	Low effectiveness against *A. castellanii* mainly in trophozoites		[30]

Table 2. *Cont.*

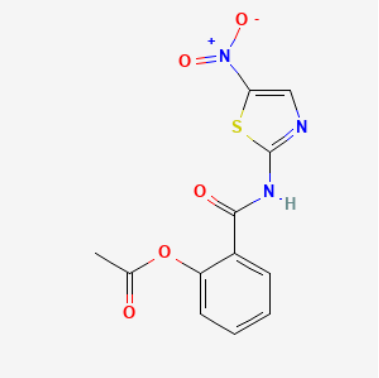
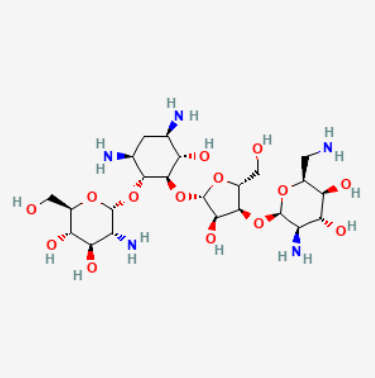
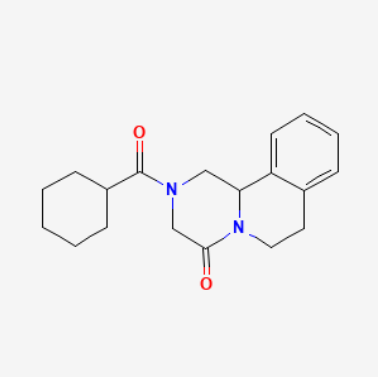
Drug of Choice	Mechanism of Action Known for Microrganisms	Descriptions and Effects on *A. castellanii*	Chemical Structure	Reference
Miltefosine	Inhibits the synthesis of important glycoproteins in the cell membrane of *Trypanosoma brucei* and *Leishmania* spp.	Induces apoptosis-like cell death, inhibiting proteinase kinase B in trophozoites and cysts		[31]
Nitazoxanide	Deprives the parasite of its energy production, inhibiting the enzyme pyruvate ferredoxin oxidoreductase of *G. lamblia* and *Cryptosporidium parvum*	Affects cellular differentiation processes, extracellular proteases, and the viability of trophozoites and cysts under microaerophilic conditions		[32]
Paromomycin	Binds to the 16S RNA portion of the ribosome, preventing protein synthesis of *E. histolytica* and *G. lamblia*	Cytotoxic activities against trophozoites and cysts		[33]
Praziquantel	Inhibits the Na^+ and K^+ pumps of schistosomes, increasing the permeability of the cell membrane and causing the weakening of the integument through the contraction and hardening of the integument	Not available		[34]

Table 2. *Cont.*

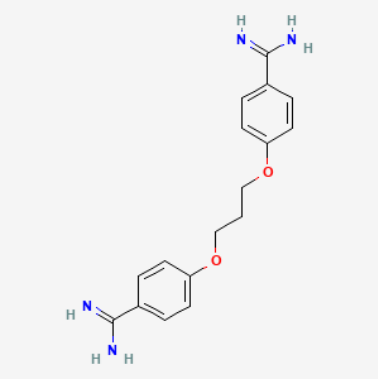
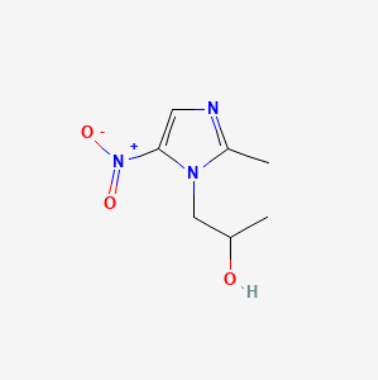
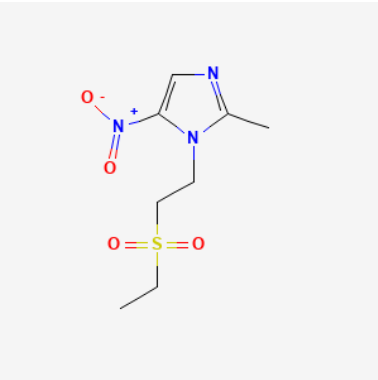
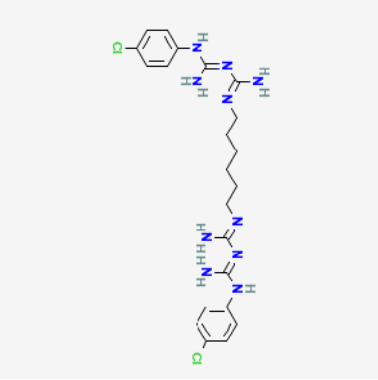
Drug of Choice	Mechanism of Action Known for Microrganisms	Descriptions and Effects on *A. castellanii*	Chemical Structure	Reference
Propamidine isethionate	Inhibits metabolic pathways or the synthesis of DNA/RNA linked to metabolic pathways of *E. histolytica*	Cytotoxic activities against trophozoites and cysts		[35]
Secnidazole	Interferes with the flow gradient of the cell membrane, altering the ability to absorb some nutrients of *E. histolytica* and *T. vaginalis*	Crosses the plasma membrane, interacting with cytoplasmic molecules of amoeba		[36]
Tinidazole	Interacts with DNA by destroying its chain or inhibiting DNA synthesis of *T. vaginalis*, *E. histolytica*, and *G. lamblia*	Not available		[37]
Antiseptic and germicidal				
Chlorhexidine	Cationic molecules of chlorhexidine react with cell wall-negative molecules of *Streptococcus mutans*	Cytotoxic activities against trophozoites and cysts		[38]

Table 2. *Cont.*

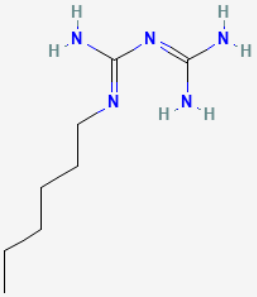
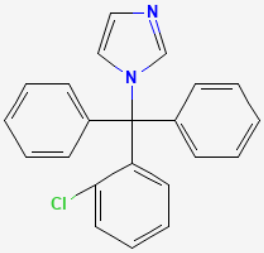
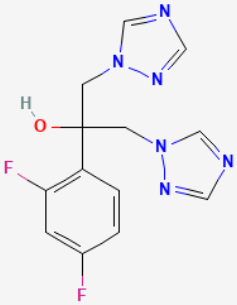
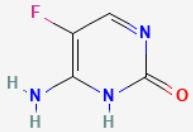
Drug of Choice	Mechanism of Action Known for Microrganisms	Descriptions and Effects on *A. castellanii*	Chemical Structure	Reference
Polyhexamethylene biguanide	Molecules of this germicide interact with the cell membrane causing pores and increasing the membrane permeability of *Candida* spp.	Impairs membrane integrity of trophozoites and cysts		[39]
Antifungal				
Clotrimazole	Damages and destroys the cell membrane, inhibiting ergosterol synthesis of *Candida* spp. and *Malassezia* spp.	Amoebicidal effects are mainly in trophozoites		[40]
Fluconazole	Inhibits ergosterol biosynthesis, damaging the cell membrane of *Candida albicans* and *Cryptococcus neoformans*	Not available		[41]
Flucytosine	Inhibits DNA synthesis and interferes with the RNA transcription of *C. neoformans*, *Fusarium* spp., and *Aspergillus* spp.	Not available		[42]

Table 2. *Cont.*

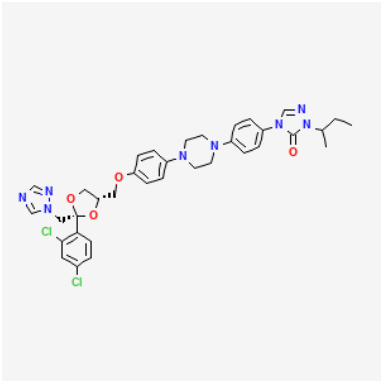
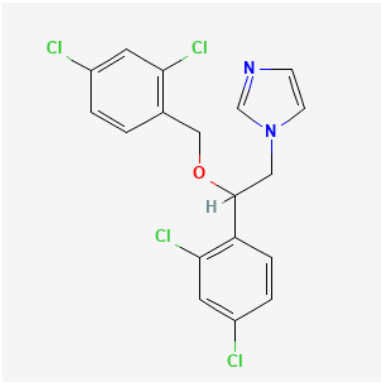
Drug of Choice	Mechanism of Action Known for Microrganisms	Descriptions and Effects on *A. castellanii*	Chemical Structure	Reference
Itraconazole	Damages the membrane of *Blastomyces dermatitidis*, *C. neoformans*, and *Histoplasma capsulatum*	Cytotoxic activities against trophozoites and cysts		[33]
Miconazole	Damages and destroys the cell membrane, inhibiting the ergosterol synthesis of *Candida* spp. and *Malassezia* spp.	Cytotoxic activities against trophozoites and cysts		[43]

Table 3. General aspects of main studies on amoebicidal drugs in vitro.

Compound	Isolate/Stage	Assays	Cell Model for Infection	IC50	Mechanism of Action and Observation	Reference
Alkyl-carbon, alkylphospho-cholines (APCs), and quaternary ammonium compounds (QACs)	Trophozoites and cysts of *A. castellanii* (ATCC 50370)	The alamar blue assay and count by hemocytometer	Not used	Octadecyltrimethyl ammonium bromide had the best IC50 17.58 µM against trophozoites and 38.21 µM against cysts	Induces membrane per-meabilization	[44]
Amphotericin B and fluconazole conjugated to silver particles	Trophozoites of *A. castellanii* (ATCC 50492)	Count by hemocytometer	HeLa cells	Amp-AgNPs and Nys-AgNPs damaged 80% of trophozoites at 10 µM. The compounds had low cytotoxicity	Mechanism of action was not evaluated	[45]
Anisomycin, prodigiosin, and obatoclax	Trophozoites of *A. castellanii* (ATCC 50370)	Count by hemocytometer	Fibroblast cells (hFF-1)	Prodigiosin and obatoclax had IC50 2.2 µM and 0.5 µM against trophozoites, respectively. The compounds had low cytotoxicity against hFF-1	Mechanism of action was not evaluated	[46]

Table 3. *Cont.*

Compound	Isolate/Stage	Assays	Cell Model for Infection	IC50	Mechanism of Action and Observation	Reference
Artemisia argyi	Trophozoites and cysts of *A. castellanii* (ATCC30010)	Count by hemocytometer	Human bronchial epithelial cells (ATCC CRL-9609)	The extract had IC50 37.4 µg/mL against trophozoites and IC50 74.8 µg/mL against cysts. The extract had high cytotoxic against human bronchial epithelial cells	Mechanism of action was not evaluated	[47]
Atorvastatin and commercial eye drops	Trophozoites of *A. castellanii* (ATCC 30010)	The alamar blue assay	Murine macrophages (ATCC J774-A1)	The best association was atorvastatin (82.4%) plus diclofenaco-lepori (17.6%), which inhibited 100% trophozoite proliferation. The compounds had low cytotoxicity murine macrophages	Mechanism of action was not evaluated	[48]
Auranofin	Trophozoites of *A. castellanii* (ATCC 30010 and JEA19)	Count by hemocytometer	Not used	*A. castellanii* strains were susceptible to auranofin and had IC50 2.97 µM–3.48 µM against trophozoites	Blocked thioredoxin reductases TrxR within the redox-active domain and disrupted the homeostasis of this system, leading to cellular oxidative stress and intrinsic apoptosis	[49]
Benzothiazole	Trophozoites and cysts of *A. castellanii* (ATCC 30010)	Count by hemocytometer	Fibroblast cell line (ATCC WI-38)	Benzothiazole had IC50 0.02% and damaged 100% trophozoites at 0.04% against trophozoites and cysts. Benzothiazole had low cytotoxicity against human fibroblast cells	Mechanism of action was not evaluated	[5]
Betulinic acid and botulin	Trophozoites and cysts of *A. castellanii* (ATCC 50492)	Count by hemocytometer	HeLa cell	Betulinic acid and botulin damaged 65% of trophozoites and 57% of cysts. Betulinic acid and botulin had low cytotoxicity against HeLa cell	Blocked the amoeba binding to human cells	[50]

Table 3. *Cont.*

Compound	Isolate/Stage	Assays	Cell Model for Infection	IC50	Mechanism of Action and Observation	Reference
Camellia sinesis	Trophozoites of *A. castellanii* (ATCC 30010)	Count by hemocytometer	Murine macrophages (ATCC J774A.1) and HCE	*C. sinesis* extract was tested against trophozoites in 25%, 50%, 75%, and 100% concentrations. *C. sinesis* extract damaged 100% of trophozoites in a concentration of 75%. *C. sinesis* extract had low cytotoxicity against HCE	Mechanism of action was not evaluated	[51]
Ammoides pusilla	Trophozoites of *A. castellanii* (ATCC 30010)	The alamar blue assay	Not used	Leaves and flower essential oil had IC50 5.32 µg/mL and aerial parts had IC50 97.18 µg/mL against trophozoites	Mechanism of action was not evaluated	[52]
Thymbra spicata	Trophozoites and cysts of *A. castellanii*	Count by hemocytometer	Not used	*T. spicata* extract damaged 100% of trophozoites at 16.0 µg/mL. The extract was not effective against cysts	Mechanism of action was not evaluated	[53]
Laurencia johnstonii	Trophozoites of *A. castellanii* (ATCC 30010)	The alamar blue assay	Murine macrophages (ATCC J774A.1)	3α-bromojohnstane extract had IC50 41.51 µM against trophozoites. Extract had low cytotoxicity against murine macrophages	Mechanism of action was not evaluated	[54]
Delphinium gracile, *D. staphisagria*, *Consolida oliveriana*, and *Aconitum napellus*	Trophozoites and cysts of *A. castellanii* (ATCC 30010)	The alamar blue assay	Vero cells (ATCC CCL-81)	Four flavonoids were effective against trophozoites and had IC50 3.5, 1.4, 1.4, and 2.3 µM and inhibited excystation. Flavonoids had low cytotoxicity against Vero cells	Mechanism of action was not evaluated	[55]
Propolis flavonoids	Trophozoites of *A. castellanii* (ATCC30010 and ATCC50739)	Count by hemocytometer	Vero cell (ATCC 84113001)	The best minimum inhibitory concentrations of the most active propolis extract against trophozoites were 62.5 µg/mL. Flavonoids had low cytotoxicity against Vero cells	Mechanism of action was not evaluated	[56]

Table 3. *Cont.*

Compound	Isolate/Stage	Assays	Cell Model for Infection	IC50	Mechanism of Action and Observation	Reference
Glimepiride, vildagliptin, and repaglinide coated in nanoparticles	Trophozoites and cysts of *A. castellanii* (ATCC 50492)	Count by hemocytometer	HeLa cells	Vildagliptin coated in silver nanoparticles damaged 80% of trophozoites at 5 µM and inhibited encystation and excystation. Vildagliptin coated in silver nanoparticles had low cytotoxicity against Vero cells	Mechanism of action was not evaluated	[57]
Histone deacetylase inhibitors	Trophozoites and cysts of *A. castellanii* (ATCC 30868)	Count by hemocytometer	HCE cells	FFK29 and MPK576 against trophozoites had IC50 4.8 and 4.7 µM, respectively, and inhibited trophozoite proliferation. Histone deacetylase inhibitors had low cytotoxicity against HCE	Mechanism of action was not evaluated	[58,59]
Irosustat and STX140 in nanoformulations	Trophozoites and cysts of *A. castellanii* (ATCC 50492)	Count by hemocytometer	HeLa cells	Irosustat NP and STX140 NP at 100 µM damaged 20% of trophozoites and inhibited excystation. Nanoformulations had low cytotoxicity against HeLa cells	Mechanism of action was not evaluated	[60]
Metformin-coated silver	Trophozoites and cysts of *A. castellanii* (ATCC 50492)	Count by hemocytometer	HeLa cells	Metformin-coated silver damaged 26.67% of trophozoite proliferation at 10 µM and inhibited encystation and excystation. Metformin-coated silver decreased *A. castellanii* cytotoxic against HeLa cells	Mechanism of action was not evaluated	[61]
Methyltrioctylammonium chloride-based deep eutectic solvents	Trophozoites and cysts of *A. castellanii* (ATCC 50492)	Count by hemocytometer	HeLa cells	DES-E had the best amoebicidal activity, damaged 85% of trophozoites at 10 µM, and inhibited encystation and excystation	Mechanism of action was not evaluated	[62]

Table 3. *Cont.*

Compound	Isolate/Stage	Assays	Cell Model for Infection	IC50	Mechanism of Action and Observation	Reference
Polyaniline-based molybdenum disulfide nanoparticles	Trophozoites and cysts of *A. castellanii* (ATCC 50492)	Count by hemocytometer	HeLa cells and primary human corneal epithelial cells	PANI/MoS2 had IC50 100 µg/mL against trophozoites and cysts. PANI/MoS2 had low cytotoxicity against HeLa cells and primary human corneal epithelial cells	Mechanism of action was not evaluated	[63]
Squaramides and acyclic polyamine derivatives	Trophozoites and cyst of *A. castellanii* (ATCC 30010)	The alamar blue assay	Vero cells (ATCC CCL-81)	Squaramides and acyclic polyamine had IC50 3.5 µM and 26.7 µM, respectively, and had complete cysticidal activity at 100 µM and 200 µM	Mechanism of action was not evaluated	[64]
Synthetic acridine-9(10H)-1	Trophozoites and cysts of *A. castellanii* (ATCC 50492)	Count by hemocytometer	Human keratinocyte cells (HaCaT cells)	Synthetic acridine-9(10H)-1 VII had IC50 53.46 µg/mL against trophozoites and inhibited excystation	Interacts with the catalytic residues and causes morphological alterations	[65]
Tannic acid-modified silver nanoparticles	Trophozoites and cysts of *A. castellanii* (P19)	The alamar blue assay	Not used	Tannic acid-modified silver nanoparticles had IC50 14 parts per million (ppm). Tannic acid-modified silver nanoparticles had low cytotoxicity	Causes a disturbance in in the plasmatic membrane, mitochondria, and nucleus	[66]
Algerian Limonium oleifolium Mill	Trophozoites of *A. castellani* strain (ATCC 30010)	The alamar blue assay	Murine macrophage (ATCC J774)	The essential oil had IC50 7.48 µg/mL against trophozoites. The essential oil had low cytotoxicity against murine macrophages	Mechanism of action was not evaluated	[67]
Ursolic acid derivatives	Trophozoites and cysts of *A. castellanii* Neff (ATCC 30010)	The alamar blue assay	Murine macrophage (ATCC J774.A1)	The two ursolic acids derivated had IC50 22.7 µM and 21.4 µM against trophozoites and IC50 18 µM and 17 µM against cysts. Ursolic acid had low cytotoxicity against murine macrophages	Mechanism of action not elucidated	[68]

Table 4. General aspects of main studies on amoebicidal drugs in vivo.

Compound	Isolate/Stage	Model Animal	Methods/Dosage/Route/Time	Results and Observations	Reference
Cationic chlorin derivative photosensitizer (TONS504) mediated photodynamic antimicrobial chemotherapy	Trophozoites of *A. castellanii* (ATCC 30868)	Male Japanese white rabbits	TONS504 was administered as eye drops at 1 mg/mL, followed by light-emitting diode irradiation after 7 days of the establishment of keratitis	58% of the rabbits recovered completely after the treatment, showing that drugs with photodynamic treatment are a good therapy	[69]
Corticosteroid eye drop	Trophozoites of *A. castellanii* (ATCC 50492)	Male Japanese white rabbits	A 26 G needle attached to a microliter syringe was advanced to the center of the cornea, and 30 µL of a suspension containing 1×10^5 trophozoites cells/mL was inoculated in the right eye, and betamethasone sodium phosphate (BSP) eye drops were administered for 5 or 7 days	Topical corticosteroids have the potential to aggravate keratitis when the cornea is infected by *A. castellanii*	[70]
Rose bengal (RB)-mediated photodynamic antimicrobial	Trophozoites of *A. castellanii*	Male New Zealand white rabbits	Rabbits were divided equally into three groups: group control without treatment and animals treated topically with two concentrations of Rose bengal (0.1% and 0.2%) associated with photodynamic treatment (+518 nm irradiation) for 5 days	RB-mediated photodynamic antimicrobial is effective in decreasing the parasitic load and clinical severity of keratitis, although this study did not perform a control with the drug of choice chlorhexidine; an association of the drug with photodynamic treatment shows good results in ocular lesions	[71]

2. Results and Discussion

2.1. Therapies Used for the Treatment of A. castellanii Infections

No specific drugs have been designed or developed to treat acanthamoebiasis owing to its varied clinical manifestations, and it is difficult to develop specific therapies [1,7,72]. Diseases caused by *A. castellanii* are treated with repurposed drugs in monotherapy or combination schemes, (Table 1) [1,7,72]. The most effective keratitis treatment agents are biguanides, with polyhexanide polyhexamethylenebiguanide (PHMB) effective at low concentrations (0.02%), despite its relative toxicity to eye cells. Chlorhexidine (0.02%) is also effective at low concentrations, but it requires the administration of other drugs, such as the aromatic diamines promazine (0.1%), propamidine isethionate (0.1%), hexamidine (0.1%), and some antibiotics (Table 1). For other clinical manifestations, a variety of drugs are used; for example, amphotericin B in combination schemes is used to treat both skin ulcers and granulomatous encephalitis (Table 1). It should be noted that clinical studies defining drug efficacy, unresponsiveness, and resistance are limited.

Table 2 lists the main drugs, antibiotics, antiparasitics, antiseptics and germicidals, and antifungals that are used in the treatment of acanthamoebiasis in clinical practice and their mechanisms of action when known.

Antibiotics are often used (Table 2). The most of mechanism of action of these drugs is not yet established, and it is possible that dysbiosis may occur in the ocular microbiota during treatment, promoting the elimination of nutrients or essential substances for amoeba and causing their death [36,56]. Azithromycin is the most used antibiotic for cases of skin

ulcers, granulomatous encephalitis, and pneumonia, while for cases of keratitis, the most used antibiotic is gatifloxacin, an antibiotic used in ophthalmological formulations.

Common antiparasitic drugs are administered for acanthamoebiasis. Owing to the lack of clinical studies, drug replacement is used, and common antiparasitic drugs, such as praziquantel, metronidazole, and tinidazole, are some of the available options [7,72]. Due to the similarity of some metabolic mechanisms between protozoans, drugs such as metronidazole and miltefosine can cause damage in *Trichomonas vaginalis, Entamoeba histolytica* and *Giardia lamblia*, and Trypanosomatids (*Trypanosoma brucei* and *Leishmania* spp.), respectively, and *A. castellanii* (Table 2).

Antifungal drugs are widely used in the treatment of parasites, as the mechanism of action of many drugs in this class is associated with the formation of pores and lysis of the cell membrane, which causes the death of fungi and works for many parasites. *A. castellanii* is no different, and clotrimazole and fluconazole (Table 2) can be used, but in cases of keratitis, such drugs can be cytotoxic to the eye cell [40,42].

It should be noted that ten out of the twenty-six dugs listed in (Table 2) do not have their mechanism of action described in the *A. castellanii* trophozoite and/or cyst. Although the mechanisms of action of drugs refer to the binding and modulation of their specific biomolecular target, the genotypic and phenotypic differences between pathogenic species such as prokaryotes, protozoa, and helminths justify the research of drug's biological effects (cell growth encystation–excystation, metabolic responses, etc.) in *A. castellanii*.

Owing to the toxicity of the drugs used for keratitis, inflammation in the corneal tissue is accentuated. The use of antiglaucoma medications to lower eye pressure is recommended [70]. Steroid drugs are not desirable options, as some studies have indicated that these anti-inflammatory drugs decrease the immune response, increase the pathogenicity and number of trophozoites, and worsen the degree of the lesions [70]. The dosage of topical ocular agents is not well established, but they are typically administered hourly for at least six months to one year, requiring the patient to administer eye drops at night, making the treatment tiring. Its discontinuation may result in *A. castellanii* resistance, aggravating the condition of the patient [70].

2.2. Therapies in Research for Pathologies Caused by A. castellanii

The general characteristics of the main studies in vitro on amoebicidal drug candidates in recent years are shown in (Table 3). Twenty out of the thirty compound studies have shown efficiency in vitro against *A. castellanii* and low toxicity to mammal cells. Of the thirty studies evaluated, the original studies had chemical substances of natural origin, plant extracts, substances from biological processes, synthetic chemical substances, microbicides, or conventional drugs (Table 3). The amount of research conducted on these drug candidates can be explained by the fact that drugs already established and studied are less costly and can advance easily to further stages of research in vitro and in vivo. As shown in Table 3, most of the mechanisms of action of these compounds are not established.

It should be noted that the use of nanocoating (five studies investigating amphotericin B, glimepiride, vildagliptin, repaglinide, metformin, tannic acid, and irosustat; Table 3) is an alternative for substances that have good amoebicidal values and high toxicity to mammal cells [57,61,63,67]. This is one of the most promising methods to treat keratitis, as the ocular tissue is an extremely sensitive region. Studies demonstrating strong cytotoxicity against human cells, such as *Artemisia argyi* methanolic extracts, which damaged bronchial epithelia cells and also had high amoebicidal activity, indicate that it would be interesting to evaluate these extracts incorporated in nanoemulsions or liposomal delivery systems [47].

It also should be noted that four out of the ten chemical substances of natural origin and plant extracts listed in Table 3 are commercially available drugs (*A. argyi*, *Camelia sinesis*, *Aconitum napellus,* and propolis).

Studies of drug association are important since one of the great clinical challenges associated with acanthamoebiasis is related to the fact that both forms of the parasite, trophozoites, and cysts, which are generally *resistant* to drugs, might be present in the

infected tissues [9]. In fact, in patients undergoing a long period of treatment, most drugs fail to act in both parasite forms, whereas the cases of relapses are associated with cystic form [53]. Thus, studies such as the one with quaternary ammonium compounds in association with alkylphosphocholines indicating an efficacy against both trophozoites and cysts are promising [44] (Table 3). The extract of *Thymbra spicata,* despite not neutralizing cysts, was toxic to the trophozoites (Table 3) and deserves further studies to evaluate its association with other drugs and compounds [53].

The glycoside flavonoids are also promising drugs since flavonoid 2 (*Delphinium gracile*) was effective against both cysts and trophozoites; this compound was also a more potent amoebicidal than chlorhexidine (a control used in a clinical trial) [55].

It can also be concluded in Table 3 that in vitro studies are not exact representations of acanthamoebiasis0associated cells since many analyses used model systems as macrophages and the immortalized Henrietta lacks cervical adenocarcinoma cells (HeLa cells), such that the choice of cells impairs the advancement of in vivo toxicity studies. Just five studies used a representative cell line for ocular HCE cells to test drug toxicity (Table 3) [50,51,58,59,63]. Thus, more studies are necessary using cell models that are representative of the *A. castellanii* infection (keratitis and granulomatous amoebic encephalitis) [50,51,58,59,63]. The primary, immortalized, and transformed HCE and cerebral cortical cells are recommended, and 3D multilayer systems reconstructed from human corneal or brain tissues should be of value in the analysis of anti-*A. castellanii* drug candidates.

Within the publication inclusion criteria for this integrative review, only three in vivo assays have been performed in the last 5 years (Table 4) [69–71], which is likely due to the ethics committee, the expensive model, and the difficulty of working with this infection model. Laboratory skills are required for the preparation of the researcher for the inoculation of the amoeba, mainly in the ocular region, a scenario that can be circumvented with more investment and training of professionals. Table 4 shows the specified medications and tests with the association of substances with photodynamic treatment, which are likely due to advances in this area of phototherapy in the treatment of cancer being used as a possible relocation for *A. castellanii* keratitis [69,71]. Also, it was demonstrated in vivo that corticosteroid eye drop instillation aggravates the development of *A. castellanii* keratitis in rabbit corneas inoculated with *A. castellanii* and bacteria (Table 4) [70]. The model animal most often used in studies on ocular infection is the rabbit because it is a better-managed animal and supports intraocular infection [69–71]. As shown in Table 4, in rabbits, rose bengal-photodynamic therapy effectively decreased the parasite load and clinical severity of keratitis [69,71]. Some alternatives models for rabbits that can help in the study of keratitis by *A. castellanii* include the zebrafish (*Danio rerio*), a popular model that is used to study vision and human ocular diseases. *Drosphila* and planarians as potential model systems should also be considered for acanthamoebiasis studies [73].

2.3. Methods that Are Being Used to Research New Drugs for A. castellanii Infection

A. castellanii is a parasite that is relatively easy to cultivate in the laboratory, and its trophozoite form is cultivated in a liquid axenic medium peptone and yeast extract (PYG) and supplemented with glucose [74]. Thophozoites turn into cysts when deprived of nutrients or cultivated on a Neff medium [44,74]. They can be viewed using ordinary light microscopy stained with Giemsa or through an inverted microscope (Figure 1). As observed in Table 3, the methods used to study amebicide drugs can be divided into qualitative and quantitative analyses.

For qualitative methods, morphological alterations, such as the destruction of organelles present in the cytoplasm (e.g., mitochondria) are analyzed [75]. The main qualitative methods used are optical, fluorescence, and electron microscopy [76]. Ordinary optical microscopy allows the analysis of the confluence and morphology of amoeba. Fluorescence microscopy provides excellent results due to the autofluorescence of amoeba; if the drug destroys the amoeba membranes, the intensity of fluorescence emitted by the amoeba with the treatment set is higher than the control group of live amoebae. Fluorescence emission

can be analyzed using a microscope or microplate reader [75]. Electron microscopy, which allows the analysis of amoeba organelles at a higher magnification than ordinary optical microscopy, provides a more refined morphological analysis, allowing for the study of organelles in which the drug acts [77].

For quantitative methods, cell viability can be analyzed by counting dead cells stained with trypan blue using a hemocytometer. Cell viability tests using methyl-thiazolyl-tetrazolium (MTT), an alamar blue assay, fluorescence, and optical microscopy can also provide quantitative results [9,49,52,57].

Although all these methods are valid for evaluating the number and morphology of A. castellanii, it is important to standardize the protocols and adapt them for use with trophozoites and cysts. Furthermore, the standardization of the methodology will allow the comparison of results obtained by different research groups working with drug candidates.

2.4. Overview of New Drug Possibilities for the Future

The investigation of therapeutic alternatives for acanthamoebiasis is difficult because of the high investment required to discover new molecules. Chemical synthesis laboratories have not developed new drugs in recent years, and despite the annual synthesis and studies of several molecules, only a few drugs have been studied in rabbits [7,52,78].

Studies are directed toward the most prevalent pathogens, as the most prevalent diseases will bring a greater financial return to financial investments toward the production of novel therapeutic forms [7,50]. Therefore, for parasitic diseases, such as those caused by *A. castellanii*, investments in the research of new therapies have become more difficult to obtain [7,50]. Thus, researchers require skills in developing cheaper stages and methods of investigation and more expansive discussions on the elaboration of experimental designs [7,79].

The bioinformatics field may assist in the development of new drugs by identifying promising agents by screening molecules in computational models because of the volume of data attainable through biocomputational platforms [7,50]. Future drugs for acanthamoebiasis caused by *A. castellanii* may be related to the reallocation of existing drugs or the biosynthesis of conventional pharmaceutical agents, or novel drugs created from plants may be developed [80,81]. There are promising results from the extracts of *Laurencia johnstonii* and *Camellia sinesis,* which showed significant activity, and all concentrations showed an inhibition of *A. castellanii* encystation for the extracts of *C. sinesis.* Moreover, the cytotoxic test showed that the extract from these plants had a low effect against macrophage and human primary corneal stromal cells, respectively [52,54].

Immunological studies should not be ruled out for cases of keratitis. It is known that monoclonal antibodies are highly effective in several lines of treatment, such as autoimmune diseases and cancer [82], and because of their high specificity and efficacy for keratitis, it may be possible to develop immunotherapeutic ophthalmic solutions [7,78,79,83].

The biological study of *A. castellanii* will be important for understanding amoeba encystment processes and gene modulation, as well as its biochemical cascade processes, ability to adhere, and phagocytosis, leading to novel therapeutic schemes [7,50].

3. Methods

For this review, original scientific articles related to pharmacological approaches against *A. castellanii* infections were collected from the following literature databases: Electronic Library Online (SciELO), PubMed, Medical Literature Analysis and Retrieval System Online (Medline), Latin American and Caribbean Literature in Health Sciences (Lilacs), and Google Academic and Capes Periodical Portal. All descriptors were selected in English and Portuguese and included "Antiparasitic Agents anti-*Acanthamoeba castellanii*," "Drug Therapy anti-*Acanthamoeba castellanii*," "Pharmacology anti-*Acanthamoeba castellanii*," "New therapy anti-*Acanthamoeba* castellanii", "Organism Model for *A. castellanii*" Antiparasitários anti-*Acanthamoeba castellanii*", "Tratamento farmacológico anti-*Acanthamoeba castellanii*", "Farmacologia anti-*Acanthamoeba castellanii*", and "nova terapia anti-*Acanthamoeba castel-*

lanii", and " Organismo modelo para *A. castellanii*". Inclusion criteria were indexed articles with publication dates from 2018 to 2023 published in English and Portuguese. Articles published before and after the stipulated dates were excluded. Based on the established criteria, 30 articles were chosen for our analytical review.

4. Conclusions

The treatment of acanthamoebiasis is complex, and current drugs are not specific. Due to the relative rarity of various clinical manifestations, clinical studies defining drug efficacy, unresponsiveness, and resistance are limited. In general, studies have shown satisfactory results of the drug candidates tested against the trophozoites, but they fail to have or evaluate the effect on the cystic form, and little is known about the mechanism of action of these options. Tests with plant extracts have shown promise in this regard, although additional in vitro and in vivo studies on their mechanism of action, toxicity, and efficiency are still highly needed. In addition, in vitro studies have little representation since most of the analysis used model systems for toxicity assays, such as macrophages and the HeLa cells, which are not representative of the infections caused by *A. castellanii* (for example, keratitis). Although there are a number of in vitro analyses and new formulations for conventional drugs, there have been few advances in in vivo studies. Additional analyses are necessary using infection model systems that are more representative and associated with acanthamoebiasis. Through this integrative review, it was possible to conclude that in vitro and in vivo studies lack translation for humans. Efforts should be made to expand the model systems used, standardize tests for evaluating anti-*A. castellanii* drug candidates, and increase and support research groups focusing on the biology of *A. castellanii* and the pharmacology of acanthamoebiasis.

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