Annals of Medical Research
DOI: 10.5455/annalsmedres.2018.07.145 2018;25(4)709-15

Original Article

Investigation of in vitro amoebicidal activities of Ornithogalum sigmoideum on *Acanthamoeba castellanii* cysts and trophozoites

Bulent Kaynak[1], Zeynep Koloren[1], Ulku Karaman[2]

[1]Ordu University, Faculty of Arts and Sciences, Department of Molecular Biology and Genetics, Ordu, Turkey
[2]Ordu University, Faculty of Medicine, Department of Medical Parasitology, Ordu, Turkey

Abstract

Aim: In this study, amoebicidal activity of methanol and ringer extracts obtained from *Ornithogalum sigmoideum* against *Acanthamoeba castellanii* cyst and trophozoite forms was investigated by determining cell viability percentage and IC_{50} values.

Material and Methods: The in vitro amoebicidal activities methanolic (1%) and Ringer extracts of *O. sigmoideum* prepared at varying concentrations (in the range of 40 mg/ml -1.25 mg/ml) on *A. castellanii* trophozoites and cysts were investigated.

Results: IC_{50} results observed at 1st, 3rd, 6th, 8th, 24th, 48th and 72nd hours in *A. castellanii* trophozite form were 23.2, 15.6, 12.1, 10.1, 8.3, 6.6 and 2.9 mg/ml by using the methanolic extract of *O. sigmoideum*, and 24.2, 16.7. 13.3, 11.9, 8.9, 8.4 and 7.3, mg/ml by using the Ringer extract ofsigmoideum respectively. The 40 mg/ml methanolic extract of O. sigmoideum killed all trophozoites at 48th and 72nd hours. The % viability rate at 72nd hour when using the Ringer extract of *O. sigmoideum* was 2.0±0.0.

Conclusion: The methanolic extract was found to be more effective than the Ringer extract on Acanthamoebatrophozoites. The sensitivity of *A. castellanii* cysts to both extracts was not very different from each other, and the sensitivity of trophozoites to both extracts was higher than that of cysts. The methanolic extracts of *O. sigmoideum* can be used as an alternative treatment option or in combination with other therapies for treating *Acanthamoeba* infections. In addition, Ringer extract of *O. sigmoideum* can also be used as an alternative to the methanolic extract.

Keywords: *Acanthamoeba castellanii;* Amoebicidal effect; *Ornithogalum Sigmoideum.*

INTRODUCTION

Amoeba living free in nature has been the focus of research in the fields of medicine and veterinary medicine since 1960 since they cause lethal parasitoses in humans and animals (1). *Acanthamoeba* species are among the common protozoa found in the environment. They have a wide distribution around the world. Habitats of *Acanthamoeba* species include soil, dust, mud, air, thermal springs, sea water, swimming pools, sewage, air-cleaning units, tap (municipal) water and bottled water, hospital environment, dental treatment units, dialysis units, contact lenses, lens storage containers, lens cleansing solutions, mammalian cell cultures, bacterial and fungal growth mediums (2-7). In addition, *Acanthamoeba* species have been isolated from plants, animals including fish, amphibians, reptiles and mammalians, from nasal mucosa, pharynx and thorax of apparently healthy individuals, infected brain and lung tissues, skin lesions of immunocompromised individuals, and corneal tissues of patients with keratitis (7-10). The risk of pathogenicity is higher in immunocompromised individuals, and in case of AIDS, cancer, previous organ transplantation, immunosuppressant use, inadequate nutrition and continuous stress (7,11).

Acanthamoeba species have two stages in their life cycle: The first stage is the trophozoite form, which actively feeds, grows, moves and multiplies; and the second stage is the cyst form, which is more resistant to the unfavorable environmental conditions (1,12). The size of the trophozoite and cyst form varies between species. When environmental conditions are appropriate, the cyst form becomes a trophozoite (7,13,14). Major diseases caused by Acanthamoeba species are Acanthamoeba keratitis (AK), which is especially seen in contact lens users, and the highly lethal granulomatous amebic

Received: 27.07.2018 **Accepted:** 14.09.2018 **Available online:** 24.09.2018
Corresponding Author: Zeynep Koloren, Ordu University, Faculty of Arts and Sciences, Department of Molecular Biology and Genetics, Ordu, Turkey, **E-mail:** zeynep.koloren@yahoo.com

encephalitis (GAE) and cutaneousacanthamoebiasis. In addition, various widespread infections that are seen in immunocompromised patients, skin lesions and pneumonia are other diseases caused by *Acanthamoeba* spp. Since *Acanthamoeba* species function as vectors for transmission of many viral and bacterial diseases including those that are caused by *Legionella pneumophila, Pseudomonas aeruginosa,* and *Mycobacterium avium*, they are clinically important (7,15-17).

Current treatment options for Acanthamoeba infections require a long time and cause some side effects, and also cyst forms that are seen in chronic infections can be resistant to drugs. In order to eliminate such problems alternative treatment methods are needed. There is an increasing tendency in modern medicine and pharmaceutical industry to make use of plant-based chemicals for this purpose (18,19).

The number of plants which are used for medicinal purposes in Turkey is estimated to be around 500-1,000. In addition, around 200 medicinal and aromatic plants have been reported to have potential for export (20,21).

As in all other countries, plants of medicinal importance in Turkey have long been used to treat diseases in the forms of tea or spices. These plants are also commonlyused in various dyes, ornaments, fragrance and flavor industries, perfumes, food additives, cleaning products and cosmetics, and new benefits are discovered by each day (22-27).

In this study, we investigated the anti- amoebic activities of methanolic and Ringer extracts of *Ornithogalum sigmoideum* plant on *Acanthamoeba castellanii* cyst and trophozoite forms by determining cell viability percentage and IC_{50} values.

MATERIAL and METHODS

Preparation of plant extract

O. sigmoideum plant, which is known as 'sakarca' by local people, was obtained from a district bazaar in the city of Ordu. The leaves, flowers and stem parts of the plant was washed with sterile deionized water in laboratory, and then dried with blotting paper. Sixty grams of samples from the stem, leaf and flower parts of the plant were weighed in an analytical scale, and grinded in 250 ml of methanol. The resulting mixture was mixed in a shaker microclimate device for 72 hours. At the end of this time, the mixture was filtered through a filter paper for coarse filtration. Afterwards, the mixture was passed through a vacuum filter containing a bacterial filter (pore size: 0.22 µm) for elimination of bacteria. 200 ml of the filtrate was taken into a sterile volumetric flask, and the methanol was evaporated via evaporator. The final concentration of the *O. sigmoideum* extract was calculated as 40 mg/ml. (28)

Trophozoites

Escherichia coli (ATCC 25922) suspension was inoculated to ringer agar growth mediums. Then, *A. castellanii* (ATCC 30010) trophozoite strain was inoculated to the same mediums. The growth mediums were incubated at 26 ± 1 °C for 3 days. At the end of this time, the petri dishes were washed with sterile Ringer solution three times in a biosafety cabinet while paying attention not to harm the trophozoites. The surface was then gently swept with a sterile loop, and collected in 15 ml sterile falcon tube, and the suspension was centrifuged at 10°C and 3000 rpm for 5 minutes. Following centrifugation, the supernatant portion was discarded, and the sediment was taken into Eppendorf tubes to be used in the experiments. The sediment portion was then taken to Thoma counting chamber, and the trophozoites were counted under a light microscope. The number of trophozoites per mL was calculated. The final concentration was adjusted to $2x10^6$trophozites/ml before starting the experiment. In order to test the vitality of the trophozoite, 0.4% trypan blue dye was added to the sediment, the mixture was vortexed and allowed to stand at room temperature for 3 minutes. The mixture was put onto a slide and covered with a coverglass, and examined under 40x magnification of light microscope. The trophozoites that engulfed the dye and appear as blue were evaluated as non-viable as described previously by Malatyalı et al. (29)

Cysts

After inoculating the trophozoites into the ringer agar medium, a portion of the petri dishes were incubated at 30 ± 1 °C for 2 weeks to allow transformation to cyst forms. At the end of this period, the same procedures that were applied to the trophozoite forms were also applied to the cyst forms. The final concentration was adjusted to $2x10^6$cysts/ml prior to the experiment. The experiment was initiated with 100% viability.

Experimental Design

1 ml of the plant extract (*O. sigmoideum* 40 mg/ml) was taken and put into sterile Eppendorf tubes. Six serial dilutions were prepared to obtain different concentrations of the plant extract. The dilutions were made using 1% methanol and ringer solution. The resulting concentrations were within the range of 1.25, 2.5, 5, 10, 20, 40 mg/ml.

Testing Amoebicidal Activity of Plant Extracts on A. castellanii Trophozoites and Cysts

100 µl of *A. castellanii* trophozoite/cyst suspension was added to each sterile Eppendorf tube. Then, 100 µl from each of the previously prepared plant extract methanol (1%)/ringer solution dilutions was added. The tubes were vortexed, and then incubated at 26 ± 1 °C. Eppendorf tubes were taken out from the incubator at 1st, 3rd, 6th, 8th, 24th, 48th and 72nd hours, respectively for performing the counts. Prior to counting, cell viability was assessed by treating with 0.4% trypan blue. 20 µl of 0.4% trypan blue dye solution was added to each of the seven sterile Eppendorf tubes. Then, 20 µl of each of the 40 mg/ml, 20 mg/ml, 10 mg/ml, 5 mg/ml, 2.5 mg/ml, and 1.25 mg/ml dilutions series, and control solution (contains only methanol (1%)/ringer solution and *A.castellanii* (trophozoite/cyst) suspension but no plant extract) was added to the tubes, and vortexed. After allowing it to stand for 3 minutes at room temperature, the number of viable cells in each tube

was examined using a Thoma counting chamber, starting from the tube with highest concentration. Trophozoite and cyst counts were assessed separately for both ringer and methanol dilution series. All experiments were performed with three repeats, and statistical analyses were applied to determine the cell viability percentage.

Statistical Analysis

The data regarding the changes observed at 1st, 3rd, 6th, 8th, 24th, 48th and 72nd hours in control and plant extracts were entered to Microsoft Excel and SPSS programs. SPSS 18 package software was used to perform descriptive data analysis, and to generate the graphics. Data are expressed as mean ± standard deviation (SD). Comparative analyses were performed within 95% confidence interval. $p<0.05$ was accepted as statistically significant.

Cell viability % results indicating the ameboicidal activity of the extracts were analyzed with multiple comparison test (Post-hoc) in SPSS 18 software. Binary comparison of the groups was performed with Tukey's test. For calculating IC50 (plant extract concentration showing lethal effect on half of the trophozoites; 50% inhibitory concentration), logarithmic regression analysis was employed. The graph plotted in the logarithmic regression analysis was used to determine the IC_{50} value. The ameboicidal actions observed with different concentrations of plant extracts at different intervals were compared against the control cells, and expressed as percent inhibition (% cell death). IC_{50} values of plant extracts in different concentrations were determined after experiments performed in three replicates.

RESULTS

In this study, the amoebicidal actions of ringer and methanolic (1%) extracts obtained from O. sigmoideumplant on A. castellanii cysts and trophozoites were investigated by determining cell viability percentage (%) and IC_{50} values. The data are expressed as mean ± standard error and a, b, c, d, e, f were different statistical values from 72. hours, p (0.05) as shown in Table 1, Table 3.

The methanolic (1%) extract of *O. sigmoideum* in 40 mg/mlconcentration showed very strong amoebicidal effect, and no viable parasites were detected at 48th hour (Table 1, Figures 2,3). IC_{50} value of methanolic extract of the plant observed at 48th hour was 6.6 mg/ml (Table 2). The Ringer extract of *O. sigmoideum* in 40 mg/ml concentration also showed a strong amoebicidal effect with 2.0±0.0 % viability rate at 72nd hour (Table 3, Figures 5, 6). IC_{50} value of Ringer extract of the plant at 72nd hour was calculated as 7.3 mg/ml (Table 4).

Table 1. Percent (%) viability effect of *O. sigmoideum* methanol extract on *A. castellanii* trophozoites and cysts at different concentrations

Concentration	Morphological form	Experimental period						
		1. hour	3. hour	6. hour	8. hour	24. hour	48. hour	72. hour
40 mg/ml	Trophozoits	6.3±0.3	3.6±0.3	2.0±0.0	1.3±0.6	0.6±0.6	0.0±0.0	0.0±0.0
	Cyst	94.3±0.3[a]	92.3±0.3[b]	90.0±0.0[c]	89.0±0.5[d]	88.3±0.3[e]	87.0±0.5[f]	85.0±0.0
20 mg/ml	Trophozoits	63.6±0.8[a]	41.3±0.8[b]	16.3±1.2[c]	9.0±0.5	6.6±0.3	5.3±0.3	3.0±0.0
	Cyst	95.3±0.3[a]	93.6±0.3[b]	92.0±0.0[c]	91.0±0.0[d]	89.3±0.3[e]	87.6±0.3	87.0±0.0
20 mg/ml	Trophozoits	82.0±2.5[a]	74.3±3.1[b]	66.3±1.7[c]	53.3±3.1[d]	42.0±1.1[e]	28.6±1.4[f]	16.0±3.2
	Cyst	96.3±0.3[a]	94.6±0.3[b]	93.0±0.0[c]	91.3±0.3[d]	90.3±0.3[e]	88.6±0.3	87.6±0.3
5 mg/ml	Trophozoits	94.6±0.6[a]	88.6±0.3[b]	84.0±0.5[c]	83.0±0.0[d]	76.3±1.7[e]	68.6±1.2[f]	41.6±1.2
	Cyst	97.0±0.0[a]	96.0±0.0[b]	93.3±0.3[c]	92.3±0.3[d]	92.3±0.3[e]	90.6±0.3[f]	89.0±0.0
2.5 mg/ml	Trophozoits	98.0±0.0[a]	93.3±0.3[b]	90.6±0.3[c]	87.0±0.0[d]	83.0±0.5[e]	72.0±2.5[f]	52.6±1.7
	Cyst	98.0±0.0[a]	96.6±0.3[b]	95.3±0.3[c]	93.3±0.3[d]	92.6±0.3[e]	91.6±0.3[f]	90.0±0.0
1.25 mg/ml	Trophozoits	98.6±0.6[a]	95.0±0.0[b]	93.3±0.3[c]	89.6±0.6[d]	85.3±0.8[e]	78.0±1.5[f]	63.6±0.3
	Cyst	99.0±0.0[a]	97.6±0.3[b]	95.6±0.3[c]	94.6±0.3[d]	93.6±0.3[e]	91.6±0.3	90.6±0.3
Control	Trophozoits	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	99.0±0.0	98.0±0.8	97.0±0.0
	Cyst	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	99.3±0.3	99.3±0.3	99.0±0.0

The data are expressed as mean ± standard error
a, b, c, d, e, f: different statistical values from 72. hours, p (0.05)
a: 1. – 72.hours; b: 3. – 72.hours; c: 6.-72.hours; d: 8.-72.h; e: 24 – 72.hours; f: 48th - 72nd hour

Table 2. IC_{50} value of *O. sigmoideum* methanol extract on *A. castellanii* trophozoites at different concentrations

A. castellanii	Experimental period	50% inhibitor concentration (IC_{50})
Trophozoite	72	2.9 mg/ml
	48	6.6 mg/ml
	2	8.3 mg/ml
	8	10.1 mg/ml
	6	12.1 mg/ml
	3	15.6 mg/ml
	1	23.2 mg/ml

Table 3. Percent (%) viability effect of *O. sigmoideum* Ringer extract at different concentrations on *A. castellanii* trophozoites and cysts

Concentration	Morphological form	Experimental period						
		1. hour	3. hour	6. hour	8. hour	24. hour	48. hour	72. hour
40 mg/ml	Trophozoits	13.6±0.6[a]	10.0±0.5[b]	7.6±0.3[c]	4.0±0.0	2.3±0.3	2.0±0.0	2.0±0.0
	Cyst	95.0±0.0[a]	92.0±0.0[b]	91.0±0.0[c]	90.0±0.0[d]	89.6±0.3[e]	89.0±0.0[f]	87.0±0.0
20 mg/ml	Trophozoits	65.3±1.2[a]	43.3±0.3[b]	21.0±0.5[c]	9.6±0.6[d]	8.6±0.8[e]	6.0±0.5	4.0±0.0
	Cyst	96.0±0.0[a]	94.6±0.3[b]	93.0±0.0[c]	92.0±0.5[d]	91.6±0.3[e]	90.0±0.0	89.0±0.0
10 mg/ml	Trophozoits	86.0±0.5[a]	77.6±0.3[b]	72.0±0.5[c]	64.6±0.3[d]	44.3±0.3[e]	43.3±0.8	40.6±1.2
	Cyst	97.3±0.3[a]	95.6±0.3[b]	94.6±0.3[c]	92.6±0.3[d]	92.0±0.0[e]	91.6±0.3	90.3±0.3
5 mg/ml	Trophozoits	97.0±0.5[a]	89.3±0.3[b]	86.3±1.2[c]	83.0±0.5[d]	79.6±0.3[e]	76.0±0.5[f]	64.3±0.3
	Cyst	98.0±0.0[a]	96.3±0.3[b]	95.3±0.3[c]	94.0±0.5[d]	93.3±0.0[e]	92.0±0.0	91.0±0.0
2.5 mg/ml	Trophozoits	98.0±0.0[a]	93.0±0.0[b]	91.0±0.0[c]	86.6±0.3[d]	83.3±0.3[e]	79.0±0.5	75.6±0.8
	Cyst	98.3±0.3[a]	96.6±0.3[b]	96.0±0.0[c]	94.6±0.3[d]	93.6±0.3[e]	92.6±0.3	92.0±0.0
1.25 mg/ml	Trophozoits	100.0±0.0[a]	96.3±0.8[b]	93.0±0.0[c]	88.6±0.8[d]	87.3±1.2[e]	83.6±0.3	80.6±0.3
	Cyst	99.0±0.0[a]	98.0±0.0[b]	97.0±0.0[c]	95.6±0.3[d]	94.6±0.3[e]	94.0±0.0	93.0±0.0
Control	Trophozoits	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	99.0±0.6
	Cyst	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0

The data are expressed as mean ± standard error
a, b, c, d, e, f: different statistical values from 72. hours, p (0.05)
a: 1. – 72.hours; b: 3. – 72.hours; c: 6.-72. hours; d: 8.-72.; e: 24.– 72.hours; f: 48th - 72nd hour

Table 4. IC50 value of O. sigmoideum Ringer extract on *A.castellanii* trophozoites at different concentrations

A. castellanii	Experimental period	50% inhibitor concentration (IC_{50})
Trophozoite	72	7.3 mg/ml
	48	8.4 mg/ml
	24	8.9 mg/ml
	8	11.9 mg/ml
	6	13.3 mg/ml
	3	16.7 mg/ml
	1	24.2 mg/ml

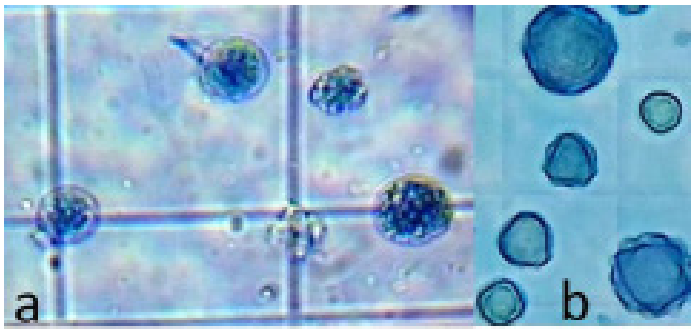

Figure 1. Image of *A. castellanii* trophozoites (a), cyst (b) that died in a light microscope (40X)

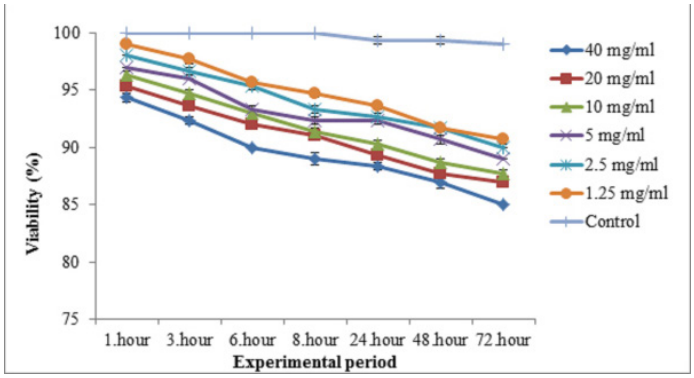

Figure 2. Diagram of amoebicidal activity on *A. castellanii* trophozoites at different times of *O. sigmoideum* prepared at different concentrations with methanol

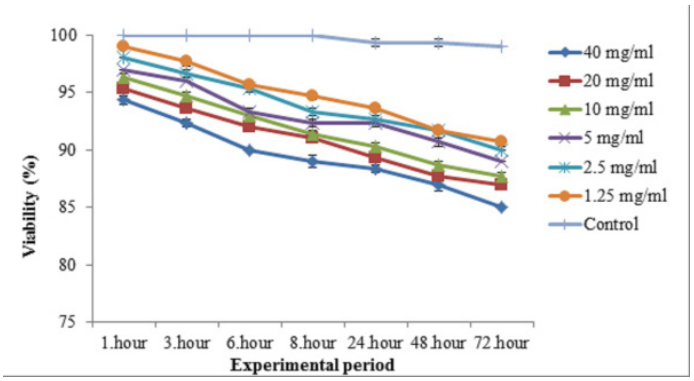

Figure 3. Diagram of amoebicidal effect on *A. castellanii* cysts at different times of *O. sigmoideum* prepared at different concentrations with methanol

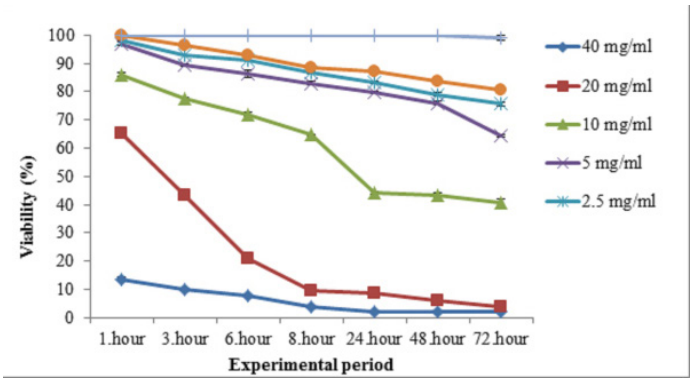

Figure 4. Diagram of amoebicidal activity on *A. castellanii* trophozoite at different concentrations of *O. sigmoideum* prepared at different concentrations with Ringer

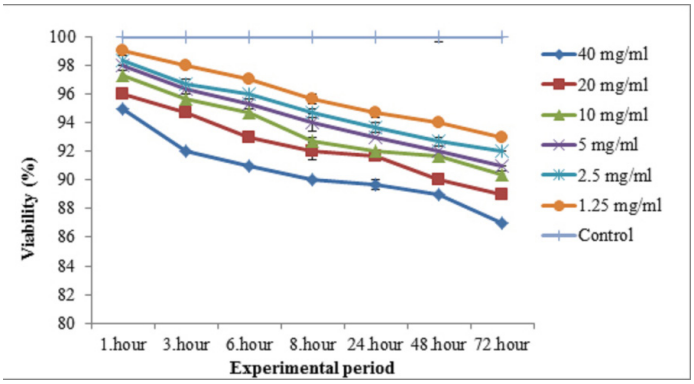

Figure 5. Diagram of amoebicidal activity on *A. castellanii* cysts at different times of *O. sigmoideum* prepared by different concentrations of Ringer

The methanolic extract of *O. sigmoideum* plant was found to have higher amoebicidal activity on Acanthamoebatrophozoites when compared to the Ringer extract of the plant. *A. castellanii* trophozites were found to be more susceptible than the cyst forms to both methanolic and Ringer extracts of *O. sigmoideum* plant. Both extracts showed similar action on the cysts (Tables 1, 3).

DISCUSSION

Currently available antiparasitic drugs may fail in treating Acanthamoebainfections as they are not sufficiently effective in certain anatomic regions including the central nervous system and the eye. Current treatment of Acanthamoeba keratitis includes cationic antiseptics such as chlorhexidine and polyhexamethylenebiguanid, either alone, or two of them combined. Since granulomatous amebic encephalitis (GAE) is seen less frequently than *Acanthamoeba* keratitis, no standard treatment scheme for GAE has been established yet (30,31). Cationic antiseptics, antifungals and macrolide group of antibiotics have been tested in vitro and in vivo for treatment, but successful results could not be achieved consistently (31,32). Some anti-amoebic drugs only have amoebistatic activity, and molecules used in the treatment can show cytotoxic effect against the host. Prolonged use of anti-amoebic drugs may not be tolerated well by the patient, and in time, microorganisms can develop resistance to these agents as well (12,30). Because of these problems, there is an ongoing search for a drug that is effective on both trophozoite and cyst forms, well tolerated and non-cytotoxic (1,31). To avoid problems due to the lack of the desired potency and selectivity of the current treatments, long treatment durations and the related side effects, and resistance development by cyst forms that are found in chronic infections, alternative treatment methods are needed. A plant-based chemical may be the best candidate for an alternative treatment modality, as there is an increasing tendency in modern medicine and pharmaceutical industry for using plant-based chemicals in treatment (18,19).

There have been a growing number of studies in the recent years on amoebicial activity of essential oils or extracts obtained from plants. Some of these studies use Acanthamoeba species to investigate the amoebicidal effect. However, there is limited information on the mechanisms responsible for the action of essential oils or plant extracts that are found to be effective.

The amoebicidal activities of various plant species on A. castellaniitrophozoite and cyst forms have been investigated in in vitro experiments using extracts prepared with methanol. Göze et al. (33) studied in vitro activity of methanolic extracts of Salvia species (*Salvia caespitosa* and *Salvia staminea*) on *A. castellanii* trophozoites and cysts, and found the methanolic extract of *S. staminea* to have more potent amoebicidal activity. Malatyalı et al. (29) evaluated the in vitroamoebicidal activities of methanolic extracts of *Peucedanum caucasicum, Peucedanum palimbioides, Peucedanum chryseum* and *Peucedanum longibracteolatum* on *A. castellanii* trophozoites and cysts, and found *P. longibracteolatum* to have the most potent amoebicidal effect. Değerli et al. (34) investigated the methanolic extracts of *Origanum syriacum* and *Origanum laevigatum* plants on *A. castellanii* trophozoites and cysts. The authors reported that *O. syriacum* showed the most potent amoebicidal activity.

Various solvents other than methanol have also been used by many researchers to prepare plant extracts. For instance, Topalkara et al. (35) compared the in vitro amoebicidal activity of the ethanolic extract of propolis on trophozoite and cysts forms of *A. castellanii,* and observed a potent amoebicidal effect on both forms. Nagwa et al. (36) evaluated the in vitroameobicidal activities of ethanolic extracts prepared from *Arachishypogaea* L., *Curcuma longa* L. and *Pancratium maritimum* L. plants on *A. castellanii* cysts, and they reported that the ethanolic extracts of A. hypogaea, C. longa and P. maritimum were more effective against Acanthamoeba cysts when compared to chlorhexidine. Badria et al. (37) examined the amoebicidal activities of ethyl-acetate and methanolic extracts of *Helianthemum lippii* (L.) on A. castellanii cysts isolated from a patient with amebic keratitis, and noted that ethyl-acetate extract was more effective than the methanolic extract of the plant. Mahboob et al. (38) studied the in vitro amoebicidal activities of ethyl-acetate, aquatic and buthanolic extracts of Lonicera japonica plant prepared using the dried flower buds. They found the ethyl-acetate extract to have the most potent amoebicidal activity.

In addition to the methanolic extract of *O. sigmoideum*, another extract was prepared from the plant using Ringer solution in the present study. In vitroamoebicidal activities of varying concentrations of methanolic and Ringer extracts of *O. sigmoideum* on trophozoite and cyst forms of *A. castellanii* were investigated. The Ringer extract was found to have lower amoebicidal activity than the methanolic extract on both trophozoite forms of *A. castellanii.*

The 40 mg/ml methanolic extract of *O. sigmoideum* killed all trophozoites at 48th and 72nd hours. While, the % viability

rate at 72nd hour at the Ringer extract of *O. sigmoideum* was 2.0±0.0.

CONCLUSION

The results of the present study demonstrate that Ringer extracts can as well be used as an alternative to the methanolic extracts. It is concluded that extracts obtained from *O. sigmoideum* under controlled conditions can be used as an alternative treatment option or in combination with other therapies for treating Acanthamoebainfections. Plant extracts examined in this study showed amobeicidal activity against both trophozoite and cyst forms of *A. castellanii.* It is recommended that whether the concentrations investigated in the present study are cytotoxic for mammalian cells, or have toxic effects on experimental animals is examined with in vivo studies, and the mechanism of action for the active substances responsible for the biological action is investigated in future studies.

Acknowledgements: The study was supported by Ordu University, Scientific Research Projects Unit (SRP), with project code BY-1705. We would like to thank SRP for their support
Competing interests: The authors declare that they have no competing interest.
Financial Disclosure: Ordu University, Scientific Research Projects Unit (SRP), with project code BY-1705.

REFERENCES

1. Saygı G, Polat Z. Free-living amoebae and their parasitosis (Primer Amibik Meningoencephalitis- Granulomatous Amibic Encephalitis - Keratitis), Cumhuriyet University Medical Faculty Review 2003;25:140-9.
2. Larkin DF, Kilvington S, Easty DL. Contamination of contact lens storage cases by *Acanthamoeba* and bacteria. Br J Ophthalmol 1990;74:133-5.
3. Gray TB, Cursons RT, Sherwan JF, et al. *Acanthamoeba,* bacterial and fungal contamination of contact lens storage cases. Br J Ophthalmol 1995;79:601-5.
4. John DT, Howard MJ. Seasonal distribution of pathogenic free-living amebae in Oklahoma waters. Parasitol Res 1995;81:193-201.
5. John DT, Howard MJ. Techniques for isolating thermotolerant and pathogenic free-living amebae.Folia Parasitol 1996;43:267-71.
6. Dağcı H, Gül S, Emre S, et al. Evaluating *Acanthamoeba* and bacterial contamination of plain contact soft contact lenses. Infect J 2001;15:357-62.
7. Madencioğlu D. Endemic Dorystoechashastataboiss., Heldr. The effect of amebicide on some *Acanthamoeba* species of ex bentham essential oil. Master Thesis. Ege University, Institute of Health Sciences, Department of Pharmaceutical Botany, Izmir 2014.
8. De Jonckheere JF, Michel R. Species identification and virulance of *Acanthamoeba* strains from human nasal mucosa. Parasitol Res 1988;74:314-6.
9. Dykova I, Lom J, Schroeder-Diedrich JM, et al. *Acanthamoeba* strains isolated from organs of freshwater fishes. J Parasitol 1999;85:1106-13.
10. Kong HH, Kim TH, Chung DI. Purification and characterization of a secretory serine proteinase of *Acanthamoeba healy*i isolated from GAE. J Parasitol 2000;86:12-7.
11. Yünlü Ö, Özçelik S, Arıcı MK. Investigation of *Acanthamoeba* and other free living amypses in swab specimens taken from the eyelids and conjunctiva. Turkey J Parasitol 2015;39:194-9.
12. Aydın E. Investigation of potency and cytotoxic activities of some members of the Salvia genus in the treatment of *Acanthamoeba castellanii*. GraduateThesis, Cumhuriyet University, Institute of Science, Department of Biology, Sivas 2008.
13. Khan NA. Acanthamoeba: biology and increasing importance in human health. FEMS Microbiol Rev 2006;30:564-95.
14. Marciano-Cabral F, Cabral G. *Acanthamoeba* spp. as agents of disease in humans. Clin Microbiol Rev 2003;16:273-307.
15. Barker J, Brown MR. Trojan horses of the microbial world: protozoa and the survival of bacterial pathogens in the environment. Microbiol 1994;140:1253-9.
16. Horn M, Wagner M. Bacterial endosymbionts of free-living amoebae. J EukaryotMicrobiol 2004;51:509-14.
17. Ertabaklar H, Türk M, Dayanır V, et al. *Acanthamoeba* keratitis due to *Acanthamoeba* genotype T4 in a non-contact-lens wearer in Turkey. Parasitol Res 2007;100:241-6.
18. Vidal F, Vidal JC, Gadelha AP, et al. *Giardia lamblia:* The effects of extracts and fractions from Mentha x piperita Lin. (Lamiaceae) on trophozoites. Exp Parasitol 2007;115:25-31.
19. Inbaneson SJ, Ravikumar S, Suganthi P. In vitro antiplasmodial effect of ethanolic extracts of traditional medicinal plant Ocimum species against *Plasmodium falciparum*. Asian Pac J Trop Med 2012;5:103-6.
20. Tarakçı S. Research on medicinal plants around Beykoz. Doctorate Thesis, Marmara University, Institute of Science, Department of Biology, İstanbul 2006.
21. Berber İ, Avşar C, Çine N, et al. Determination of antibacterial and antifungal activities of methanolic extracts of some plants grown in Sinop. Karaelmas Sci. Eng J 2013;3:10-16.
22. Ilçim A, Dığrak M, Bağcı E. Investigation of antimicrobial effects of some plant extracts. Tr J Biol 1998;22:119-25.
23. Başer KHC. The bright future of essential oils.Anadolu University Medical and Aromatic Plant and Drug Research Center, Eskişehir, Bul Med Aromat Plants 2000;20-33.
24. Dülger B, Uğurlu E, Gücin F. The antimicrobial activity of *Vitexagnus-castus* (Hayıt), Ecol Environ Rev 2002;11:1-5.
25. Dülger B, Uğurlu E, Gücin F. The antimicrobial activity of Vitexagnus-castus (Hayıt), Ecol Environ Rev 2002;11:1-5.
26. Toroğlu S, Çenet M. Uses of some plants used for therapeutic purposes and methods used for determination of antimicrobial activities. Kahramanmaras Sutcu imam University. Sci Eng Rev 2006;9:12-9.
27. Çopuroğlu Ö. Antimicrobial activities of some endemic plant species in the Niğde region.Graduate Thesis, Niğde University, Institute of Science, Department of Biology, Niğde 2013.
28. Sifaoui I, López-Arencibia A, Martín-Navarro CM, et al. Activity assessment of Tunisian olive leaf extracts against the trophozoite stage of Acanthamoeba. Parsitol Res 2013;112:2825-9.
29. Malatyalı E, Tepe B, Degerli S, et al. In vitro amoebicidal activity of four *Peucedanum* species on *Acanthamoeba* castellanii cysts and trophozoites.Parasitol Res 2011;110:167-74.
30. Fiori PL, Mattana A, Dessì D, et al. In vitro acanthamoebicidal activity of a killer monoclonal antibody and a synthetic peptide. J. Antimicrob Chemother 2006;57:891-8.
31. Ergüden C. Essential oils of Acanthamoeba spp. effect on cysts and trophozoites. Graduate Thesis, Dokuz Eylül University, Institute of Health Sciences, Department of Medical Parasitology, İzmir 2015.
32. Mattana A, Biancu G, Alberti L, et al. In vitro evaluation of the effectiveness of the macrolide rokitamycin and chlorpromazine against *Acanthamoeba castellanii*. Antimicrob Agents Chemother 2004;48:4520-7.

33. Göze I, Alim A, Dağ S, et al. In vitro amoebicidal activity of *Salvia staminea* and *Salvia caespitosa* on A*canthamoeba castellanii* and their cytotoxic potentials on corneal cells. J Ocul Pharmacol Ther 2009;25:293-8.
34. Değerli S, Tepe B, Çeliksöz A, In vitro amoebicidal activity of *Origanum syriacum* and *Origanum laevigatumon* Acanthamoeba castellanii cysts and trophozoites. Exp Parasitol 2012;131:20-4.
35. Topalkara A, Vural A, Polat Z, et al. In vitro amoebicidal activity of Propolis on *Acanthamoeba castellanii.* J Ocul Pharmacol Ther 2007;23:40-5.
36. El-Sayed NM, Ismail KA, Ahmed SA , et al. In vitro amoebicidal activity of ethanol extracts of *Arachis hypogaea* L., *Curcuma longa* L. and *Pancratium maritimum* L. on *Acanthamoeba castellanii* cysts. Parasitol Res 2012;110:1985-92.
37. Badria FA, Hetta MH, Sarhan RM, et al. Lethal effects of *Helianthum lippii* (L.) on *Acanthamoeba castellanii* cysts in vitro. Korean J Parasitol 2014;52:243-9.
38. Mahboob T, Azlan AM, Tan TC, et al. Anti-encystment and amoebicidal activity of *Lonicera japonica* Thunb. and its major constituent chlorogenic acid in vitro. Asian Pac J Trop Med 2016;9:866-71.