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Comparison of the Synergistic Effects of Black Tea and Microwave with Gentamicin and Microwave on *Acanthamoeba* cyst Mortality *In vitro*

Acanthamoeba Kistinin İn vitro Mortalite Oranı Üzerinde Siyah Çay ve Mikrodalganın Sinerjistik Etkisinin Gentamisin ve Mikrodalganın Sinerjistik Etkisi ile Karşılaştırılması

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ABSTRACT

Objective: Drug resistance in *Acanthamoeba* poses a significant challenge, prompting the need for alternative treatments. This research aimed to explore the combined treatment of chemical or phytomedicines and microwaves radiation.
Methods: The *Acanthamoeba* strain was cultivated on non-nutrient agar. Black tea extracts were prepared using the maceration method. Final concentrations of 0.75 mg/mL and 0.375 mg/mL of gentamicin and tea, respectively, were used in this study. The samples were divided into 12 groups based on drug incubation time and repeated radiation exposure, either before or after incubation. The effects of combining gentamicin and black tea extracts with microwave exposure were then evaluated on the parasite.
Results: Our results showed that the growth inhibition of *Acanthamoeba* was significantly higher in the combined treatment groups compared to gentamicin, black tea, or microwave radiation alone ($p<0.0001$-$p<0.04$). It seems that the microwave radiation led to an increasing trend in growth inhibition within 72 hours.
Conclusion: Microwave radiation can play a significant complementary role in the treatment of *Acanthamoeba* cysts by gentamicin and black tea extracts. This effect was more significant on the irradiated cysts incubated with gentamicin and also depended on the increase in incubation time and the repetition of radiation.
Keywords: *Acanthamoeba*, black tea, combination treatment, drug resistance, gentamicin, radio frequency

ÖZ

Amaç: *Acanthamoeba*'nın ilaç direnci, alternatif yeni yöntem bulma konusunda önemli bir endişe kaynağıdır. Bu araştırmada kimyasal veya bitkisel ilaçlar ile mikrodalga radyasyonunun kombine tedavisinden faydalanılmaya çalışılmıştır.
Yöntemler: *Acanthamoeba*, besleyici olmayan agar üzerinde yetiştirildi. Siyah çay ekstraktları maserasyon yöntemiyle hazırlandı. Mevcut çalışmada kullanılan gentamisinin nihai konsantrasyonları 0,75 ve 0,375 mg/mL'dir. Örnekler ilaç inkübasyon süresi ve inkübasyon öncesi ve sonrası radyasyona tekrar maruz kalma (24, 48 ve 72 saat) açısından 12 gruba ayrıldı. Gentamisin bileşiği ve siyah çay ekstraktlarının mikrodalgaya maruz bırakılmasıyla parazit üzerindeki etkileri değerlendirildi.
Bulgular: Mevcut deneyler, gentamisin, siyah çay ve radyasyon tedavisi grubuyla karşılaştırıldığında bileşik tedavi gruplarında parazitin büyüme inhibisyonunun önemli ölçüde daha yüksek olduğunu gösterdi ($p<0,0001$-$p<0,04$). Mikrodalga radyasyonunun 72 saat içinde büyüme inhibisyonunun artan bir eğilime yol açtığı görülmektedir.
Sonuç: Mikrodalga radyasyonu, *Acanthamoeba* kistlerinin gentamisin ve siyah çay ekstraktları ile tedavisinde önemli bir tamamlayıcı rol oynayabilir. Bu etkinin gentamisin ile inkübe edilen ışınlanmış kistlerde daha belirgin olduğu ve inkübasyon süresinin artmasına ve radyasyon tekrarına da bağlı olduğu görüldü.
Anahtar Kelimeler: *Acanthamoeba*, siyah çay, kombinasyon tedavisi, ilaç direnci, gentamisin, radyo frekansı

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Türkiye PARAZİTOLOJİ Dergisi

INTRODUCTION

Acanthamoeba is a free-living amoeba and one of the most common protozoa found in nature. This opportunistic parasite has been isolated from diverse environments, including soil, dust, fresh water, seawater, swimming pools, dental units, air conditioners, and hospital spaces (1). There are two phases of in *Acanthamoeba* life cycle: trophozoite and cyst. The cysts of this parasite are resistant to lethal substances like chlorine and antibiotics and can withstand a wide range of temperatures. Notably, it causes eye infections known as *Acanthamoeba* keratitis (AK) (2). If AK is not treated, it can progress to stroma damage and perforation, leading to significant vision loss and possibly blindness (3). The rising use of contact lenses has led to a significant increase in AK, particularly affecting individuals aged 15 to 25 (4,5). Despite long-term treatment, AK may not always respond. The current therapeutic approach involves using various drugs, such as gentamicin, natamycin, neosporin, broline, propamidine isothionate, and miconazole (6,7). Gentamicin, frequently used for treating ocular keratitis, exerts its antibacterial effect by disrupting bacterial cell membranes and inhibiting protein synthesis. It is applied as eye drops to treat conditions like conjunctivitis, corneal ulcers, keratoconjunctivitis, and inflammation caused by microorganisms, including *Acanthamoeba* (8).

Scientific research continues to seek effective, low-risk treatments for infectious and non-infectious diseases (9,10). Herbal medicine is widely regarded as a potential alternative for treating parasitic diseases (9). Tea has long been used as an herbal remedy, prepared by brewing leaves, buds, or stems from various plants. In Iran, over 20 types of tea are made using different plant parts, each with unique properties in traditional medicine (11). In Iran, the most common teas are green, black, white, and sour tea. Green and black tea are derived from the *Camellia sinensis* plant (11). Black tea is richer, stronger, and more bitter than green tea due to the complete oxidation and drying of its leaves during processing (12). Black tea is rich in caffeine, theophylline, L-theanine, and antioxidants like catechins, while containing minimal fat, carbohydrates, and protein (13,14). Numerous studies have examined tea's effects and mechanisms in treating various diseases. One study found that tea can help prevent obesity, diabetes, metabolic syndrome, and cardiovascular diseases (15). Research has confirmed that both green and black tea inhibit oxidation (16). Studies also show that tea catechins can block toxins from bacteria like *Escherichia coli* and reduce its pathogenicity (17,18). Experimental studies suggest that high concentrations of tea may serve as an adjunct therapy alongside antibiotics (19,20).

The use of radio frequency electromagnetic waves, both *in vitro* and *in vivo*, is a novel method being explored for disease treatment. Radio waves, known for their high frequency, can penetrate tissue, generate heat, and act quickly. Modern life is continuously exposed to microwave radio waves from devices like mobile phones and microwave ovens (21,22). While many researchers study the harmful effects of waves, others have explored their potential to kill pathogens and treat diseases, making significant progress (23-25). Recent studies show that high-intensity microwaves from ovens, mobile phones, and other devices can increase the mortality of protoscolices, hydatid cyst walls, and *Acanthamoeba* cysts (26-29).

It is essential to create innovative approaches that utilize combination therapies to tackle the deficiencies of conventional methods, ultimately aiming to lessen the complications of infectious keratitis and boost clinical effectiveness. This study aims to examine the synergistic effects of microwave irradiation combined with tea extract or gentamicin on the mortality of *Acanthamoeba* cysts. While previous research has investigated the impact of microwaves from devices like cell phones and ovens on parasites (9,30), the combined effects of microwaves with tea extract or gentamicin on *Acanthamoeba* remain unexplored. This study builds on prior research that demonstrated the antimicrobial potential of microwaves and the therapeutic properties of tea extracts. It explores an innovative combination therapy aimed at overcoming the limitations of existing treatments, reducing complications associated with *Acanthamoeba* infections, and improving clinical outcomes. By examining the interactions between microwave irradiation and tea extract or gentamicin, this research seeks to provide insights into novel and more effective treatment strategies for AK.

METHODS

Parasite Preparation

Acanthamoeba strain MG066681 (T4 genotype), kept in Parasitology Laboratory of Arak University of Medical Sciences was used for this study. This *Acanthamoeba* strain used in the study was an environmental (soil) (31). The parasite was cultured on non-nutrient agar plates coated with killed *Escherichia coli* at laboratory temperature (28 °C) (32). After 1-3 weeks, the surface of the plate was washed with sterile Page's saline, and the cysts were collected and concentrated by centrifugation at 1500 × g for 5 minutes (33). The cysts washed 3 times with phosphate-buffered saline (PBS). The viable and non-viable cysts were counted using trypan blue staining (unstained viable and stained non-viable cyst) and by hemocytometer. The parasitic suspension in which ≥90% of the cysts were alive was used for the study. Finally, a parasitic suspension containing 2×10^5 cysts per mL was prepared for this experimental study. The amount of parasite was 4×10^4 in 200 µL of parasite suspension.

Tea Extract Preparation

Black tea (*Camellia sinensis*) was obtained from the Medical Plants Research Center, SKUMS, and transferred to the Infectious Diseases Research Center, Faculty of Medicine University of Medical Sciences. The extracts of the black tea were prepared via maceration as follows: 100 g black tea was transferred into an Erlenmeyer flask; 1 L of 70% ethanol was added and the solution was placed at the laboratory temperature for 24 h. The extract was filtered through filter paper, dried via rotary evaporation, and stored at 4 °C (34). The final concentration of tea extract was used in experiment stage was 30 mg/mL.

Drug Preparation

Gentamicin was prepared (Sina Darou, Tehran, Iran). The results of some studies have shown that a concentration of 3 mg/mL of this drug leads to complete mortality of *Acanthamoeba* cysts (35). So, in the current study, lower concentrations of this drug were used. Final concentrations of 0.75 and 0.375 mg/mL of drug used in the current study.

Microwave Generator

The device used for irradiation of microwaves was Thermatur m250, Uniphy Elektromedizin Company, made in Germany. The radiant power of the device was 250 W (antenna of the device) and a frequency of 2450 MHz with an intensity of 2150 W/m (Figure 1).

Microwave Radiation Intensity Measurement

The measurements were performed by a TES 92 device with the capability to measure the EMF frequency range of 3.5 MHz-50GHz.

Experiments

200 µL of parasitic suspension were exposed with PBS, gentamicin (final concentration 0.75 mg/mL), tea extract (final concentration 30 mg/mL) and microwave radiation (25 minutes) as control groups 1 to 4, respectively. Parasite mortality was recorded up to 72 hours at 24-hour intervals in these groups. The treatment experiments were performed by four different protocols (I to IIII). Each protocol included two treatment groups (I_1 and I_2), resulting in a total of eight treatment groups (Groups 5 to 12).

Group I: Incubation before irradiation

• **I_1:** Incubation with gentamicin, followed by repeated cycles of microwave radiation and incubation (A-F).

• **I_2:** Incubation with tea extract, followed by repeated cycles of microwave radiation and incubation (A-F).

Group II: Irradiation before incubation

• **II_1:** Initial microwave irradiation, then incubation with gentamicin, followed by alternating cycles of irradiation and incubation (G-L).

• **II_2:** Initial microwave irradiation, then incubation with tea extract, followed by alternating cycles of irradiation and incubation (G-L).

Group III: Simultaneous incubation and irradiation

• **III_1:** Microwave irradiation performed simultaneously with incubation using gentamicin, followed by alternating cycles of irradiation and incubation (M-R).

• **III_2:** Microwave irradiation performed simultaneously with incubation using tea extract, followed by alternating cycles of irradiation and incubation (M-R).

Figure 1. Microwave irradiation generator

Group IV: Double irradiation before incubation

• **IV_1:** Two microwave irradiation cycles first, followed by incubation with gentamicin and subsequent incubations (S-U).

• **IV_2:** Two microwave irradiation cycles first, followed by incubation with tea extract and subsequent incubations (S-U).

At the end of each exposure time, the cysts were washed three times by PBS to remove residual drugs. The washed cysts resuspended in 100 µL PBS and used for investigated of viable and non-viable cysts and calculating percent of eliminated cysts (or % growth inhibition) by Trypan blue exclusion assay (36,37). All experiments were repeated 3 times. After 25 min of microwave exposure, the temperatures were increased from 22.6±23 to 23.2±23.9 °C. The average temperature was 0.9 °C.

Calculation of growth inhibition percent (or % eliminated cysts)

Summary, the cyst suspension and trypan blue were mixed at 1:1 ratio. The mixture was loaded in hemocytometer and number of viable and non-viable cysts counted under light microscope. The calculation was performed as follows (36).

% viable cysts = Total number of viable cysts per mL/total number of cysts per mL × 100

% growth inhibition (or % eliminated cyst) =100-% viable cysts.

Statistical Analysis

The analysis of data was done by SPSS (version 23, SPSS/PC Inc., Chicago, IL, USA) and Excel (2016). The results were presented as mean ± standard deviation and percent of eliminated cyst (or % growth inhibition). Differences between the growth inhibitions of groups were analyzed by One-Way ANOVA test. Statistical significance was defined as $p<0.05$.

Ethical Approval

All experiments were approved by Ethical Committee of the Arak University of Medical Sciences in this research (number: REC.1400.198, date: 2021-10-31).

RESULTS

The assessment of growth suppression, along with the survival and viability rates of *Acanthamoeba* in the control groups, was conducted, and the results for each group are presented in Table 1.

The treatment groups were shown in the Table 2a. The incubation time was 24 h and the irradiation time was 25 minutes in all treatments.

The levels of growth inhibition, along with the viability and survival rates of *Acanthamoeba* in the treatment groups (5-12), were evaluated. The findings for each treatment phase (A-U) are detailed in Table 2b. It should be noted that the parasite survival number is expressed as $\times 10^{-4}$.

An analysis of the 12 groups based on the duration of exposure revealed that all treated groups (groups 5 to 12) exhibited significantly greater growth inhibition compared to the control groups (groups 1 to 4) ($p<0.05$), except for group 2 (gentamicin control) and group 9 (Table 2b).

The growth inhibition rates of the treatment groups (gentamicin and tea) under protocols I to IV are presented in Figure 2.

Table 1. Growth inhibition and viable cyst percentages of *Acanthamoeba* in control groups

	Group 1			Group 2		
Exposure time (h)	**□ Survival cyst/mL mean ± SD**	**% viability mean ±SD**	**% growth inhibition**	**□ Survival cyst/mL mean ± SD**	**% viability mean ± SD**	**% growth inhibition**
24	20±0	100±0	0	14.6±0.06	73±0.30	27
48	20±0	100±0	0	14.2±0.07	71±0.35	29
72	20±0	100±0	0	13.8±0.08	69±0.39	31
	Group 3			**Group 4**		
Exposure time (h)	**□ Survival cyst/mL mean ± SD**	**% viability mean ±SD**	**% growth inhibition**	**□ Survival cyst/mL mean ± SD**	**% viability mean ± SD**	**% growth inhibition**
24	20±0	100±0	0	19.2±0.07	96±0.2	4
48	19.8±0.03	99±0.17	1	18.8±0.05	94±0.25	6
72	19.8±0.04	99±0.2	1	18.2±0.05	91±0.24	9

Group 1: PBS, Group 2: Gentamicin, Group 3: Tea extract, Group 4: Microwave, SD: Standard deviation □: × 10^{-4}

Table 2a. The treatment groups and the stages of treatment

Treatment groups	Treatment subgroups	Protocols
5	**$I_{1:}$** Parasite suspension + gentamicin (final concentration: 0.75 mg/mL)	**I** First, the *Acanthamoeba* cyst suspension was incubated with gentamicin or tea for 24 hours (**A**), followed by microwave irradiation (25 min) according to the protocol (*).
6	**$I_{2:}$** Parasite suspension + tea extract (final concentration: 30 mg/mL)	
7	**$II_{1:}$** Parasite suspension + exposure then adding gentamicin (final concentration: 0.75 mg/mL)	**II** First, the *Acanthamoeba* cyst suspension was exposed to microwave irradiation (25 min) (**G**), followed by a 24-hour incubation with gentamicin or tea. The samples were then irradiated again following protocol (**).
8	**$II_{2:}$** Parasite suspension + exposure then adding tea extract (final concentration: 30 mg/mL)	
9	**III_1**: Parasite suspension + gentamicin (final concentration: 0.75 mg/mL) + microwave irradiation (25 min)	**III** *Acanthamoeba* cyst suspension was exposed to simultaneous microwave irradiation (25 min) and 24-hour incubation with either gentamicin or tea (**M).** The samples were then irradiated again following protocol (***)
10	**III_2:** Parasite suspension + tea extract (final concentration 30 mg/mL) + microwave irradiation (25 min)	
11	**IV_A:** Parasite suspension + double microwave irradiation followed by the addition of gentamicin (final concentration: 0.75 mg/mL)	IV Parasites undergo two consecutive rounds of irradiation instead of one (25 min), followed by incubation with gentamicin or tea for 24 hours (**S**), and then the incubation protocol (****) is applied.
12	**IV_B:** Parasite suspension + double microwave irradiation followed by the addition of tea extract (final concentration: 30 mg/mL)	

* **B:** 25 minutes microwave radiation**, C:** 24-hour incubation, **D:** 25 minutes microwave radiation**, E:** 24-hour incubation**, F:** 25 minutes microwave radiation
** **H:** 24-hour incubation with tea or gentamicin, **I:** 25 minutes microwave radiation, **J:** 24-hour incubation, **K:** 25 minutes microwave radiation, **L:** 24-hour incubation
*** **N**: 25 minutes microwave radiation, **O:** 24-hour incubation, **P:** 25 minutes microwave radiation, **Q:** 24-hour incubation, **R:** 25 minutes microwave radiation
**** **T:** 24-hour incubation, **U:** 24-hour incubation

Table 2b. Inhibition effects and percent of viable *Acanthamoeba* cysts treated at stages of protocols I-IV

Process	Protocol I						Process	Protocol II					
	Group 5*			Group 6*				Group 7**			Group 8**		
	Survival cyst/mL mean ± SD	% viability mean ± SD	% growth inhibition	Survival cyst/mL mean ± SD	% viability mean ± SD	% growth inhibition		Survival cyst/mL mean ± SD	% viability mean ± SD	% growth inhibition	Survival cyst/mL mean ± SD	% viability mean ±SD	% growth inhibition
A	14±0.03	70±0.17	30	17.8±0.02	89±0.1	11	G	11.8±0.08	59±0.4	41	17.6±0.03	88±0.15	12
B	12±0.034	60±0.17	40	17.2±0.08	86±0.4	14	H	10±0.06	50±0.3	50	17.4±0.03	87±0.15	13
C	12±0.03	51±0.3	49	16±0.03	80±0.15	20	I	7.8±0.03	39±0.15	61	17±0.04	85±0.2	15
D	8.6±0.03	43±0.15	57	15.6±0.01	78±0.05	22	J	6.2±0.03	31±0.15	69	16.6±0.02	83±0.1	17
E	7.6±0.03	38±0.15	62	15.2±0.02	76±0.1	24	K	5.2±0.02	26±0.1	74	16.4±0.04	82±0.2	18
F	7.2±0.08	36±0.4	64	15±0.03	75±0.15	25	L	4.8±0.04	24±0.2	76	16±0.03	80±0.15	20
			Protocol III							Protocol IV			
		Group 9***			Group 10***				Group 11****			Group 12****	
Process	□Survival cyst/mL mean ± SD	% viability mean ± SD	% growth inhibition	Survival cyst/mL mean ± SD	% viability mean ± SD	% growth inhibition	Process	Survival cyst/mL mean ± SD	% viability mean ± SD	% growth inhibition	□Survival cyst/mL nean ± SD	% viability mean ± SD	% growth inhibition
M	17.4±0.04	87±0.2	13	16.2±0.01	81±0.05	19	S	13±0.02	65±0.1	35	16.6±0.03	83±0.15	17
N	16.4±0.02	82±0.1	18	16.2±0.03	81±0.15	19	T	10.8±0.04	54±0.2	46	16±0.02	80±0.1	20
O	16.2±0.02	81±0.1	19	15.8±0.01	79±0.05	21	U	7.6±0.04	38±0.2	62	15.6±0.03	78±0.15	22
P	14±0.03	70±0.15	30	15.6±0.01	78±0.05	22							
Q	13.6±0.03	68±0.15	32	15.6±0.04	78±0.2	22							
R	13.6±0.01	68±0.05	32	15.4±0.02	77±0.1	23							

*: Parasite was incubated with control drug or tea prior to microwave exposure
**: Parasite exposure to microwaves prior to incubation with control drug or tea
***: Simultaneous exposure of parasites to microwaves and control drug or tea treatment and followed by incubation
****: parasite exposure to double irradiation microwave before incubation with control drug or tea
A: 24-h incubation with control drug or tea, **B:** 25 minutes microwave radiation, **C:** 24-hour incubation, **D:** 25 minutes microwave radiation, **E:** 24-hour incubation, **F:** 25 minutes microwave radiation,** **G:** 25 minutes microwave radiation, **H:** 24-h incubation with control drug or tea, **I:** 25 minutes microwave radiation, **J:** 24-hour incubation, **K:** 25 minutes microwave radiation, **L:** 24-hour incubation
*** **M:** Simultaneous exposure microwaves (25 min) and control drug or tea treatment followed by 24-h incubation, **N:** 25 minutes microwave radiation, **O:** 24-hour incubation, **P:** 25 minutes microwave radiation, **Q:** 24-hour incubation, **R:** 25 minutes microwave radiation
****S.** Double irradiation microwave followed by, 24-h incubation with control drug or tea, **T:** 24-hour incubation, **U:** 24-hour incubation
□: $\times 10^{-4}$, SD: Standard deviation

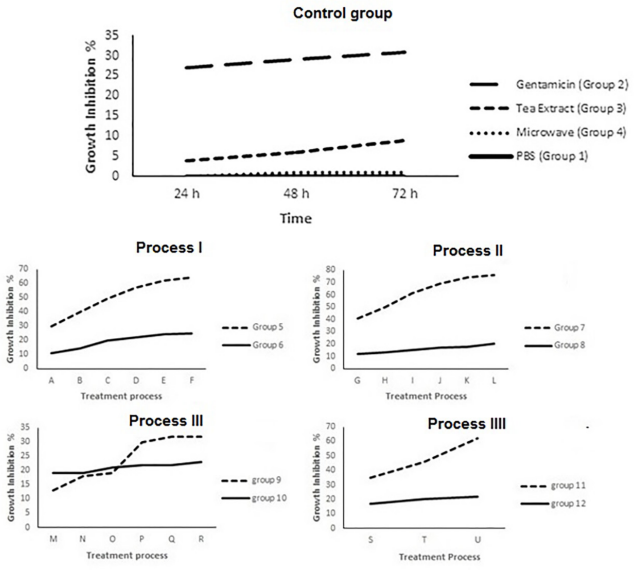

Figure 2. Comparison of *Acanthamoeba* growth inhibition across groups

DISCUSSION

This study revealed that microwave radiation (250 W, 25 minutes) alone was ineffective in achieving significant mortality in *Acanthamoeba* cysts. The lack of effectiveness can be attributed to insufficient moisture within the parasites to induce intracellular oscillations and a negligible increase in temperature during treatment. Studies have shown that the thermal and non-thermal effects of microwaves are heavily dependent on the water content of the target organism. Zhang et al. (23) reported that moist environments significantly enhance the efficacy of microwave radiation against microbial cysts by facilitating intracellular heating. Previous findings emphasize that effective microwave treatments require higher moisture content and elevated temperatures to deactivate *Acanthamoeba* cysts, as demonstrated by studies reporting complete cyst mortality at higher power settings and prolonged exposure times (22,30).

Among the treatment protocols tested, the conventional drug Gentamicin in protocol II demonstrated the highest growth inhibition rate, particularly in Group 7. Gentamicin's efficacy can be attributed to its established bactericidal mechanism, targeting the bacterial-like ribosomes of *Acanthamoeba* (15). In contrast, the tea extract in protocol III initially exhibited a greater inhibitory effect on parasite growth than gentamicin. This effect is likely due to the bioactive compounds in tea, such as catechins and tannins, which disrupt cellular membranes and metabolic pathways (9). By the end of the experiment, however, the tea extract's effectiveness became comparable to other protocols, suggesting a transient potency that diminishes over time.

A comparative analysis of Protocols II and III indicates that increasing the frequency of radiation exposure significantly enhanced growth inhibition, particularly in Group II. This aligns with Shaw et al. (38), who demonstrated that the non-thermal effects of pulsed microwave radiation effectively inactivated *Escherichia coli* and *Staphylococcus aureus*, potentially boosting the efficacy of concurrent treatments. A study investigated the use of gold nanoparticles combined with microwave radiation as an antiparasitic treatment. The results showed that increased microwave exposure time in the presence of gold nanoparticles led to a significant decline in parasite survival rates, indicating a synergistic effect that enhances growth inhibition (39). A research demonstrated that microwave can significantly enhance the sensitivity of cancer cells to anticancer drugs (40). This suggests that microwave exposure can improve the efficacy of co-administered therapeutic agents, potentially applicable to antiparasitic treatments.

However, the results indicate that while microwave exposure aids growth inhibition, gentamicin remains more effective overall than the tea extract. This observation is consistent with earlier findings that highlight the superior efficacy of conventional drugs over botanical alternatives in some contexts (41,42).

The combination of microwave radiation with gentamicin or tea extract yielded mixed results. In groups 9 and 10, the initial 48-hour period showed that tea extract combined with microwave radiation exhibited a stronger inhibitory effect on *Acanthamoeba* than gentamicin. This finding suggests a potential synergistic effect between microwave radiation and tea extract during the early stages of treatment. Synergistic effects may arise from microwave-induced structural weakening of the cyst wall, enhancing the penetration of bioactive compounds (43).

Comparison of protocols III with II shows that microwaves reduced drug efficiency to less than half that of protocols II. However, the overall inhibition rates in groups 11 and 12 were similar to those in groups 5-8, suggesting an inconsistent synergistic effect over time. This variability may be influenced by factors such as treatment sequence, exposure duration, and parasite heterogeneity.

Exposure timing also appeared to have limited impact on tea treatment group. Early or later exposure of the parasites to microwave radiation relative to the tea extract did not significantly enhance growth inhibition. This finding contrasts with studies emphasizing the importance of treatment timing in maximizing synergistic effects. Cheng et al. (44) demonstrated that pre-treatment with microwaves followed by drug exposure increased efficacy in bacterial biofilms by disrupting extracellular matrices.

Previous studies have shown that tea extracts and electromagnetic waves individually have anti-*Acanthamoeba* effects. Hajihossein et al. (9) found that green and black tea extracts were more effective than the anti-keratitis drug Natamycin, with efficacy depending on incubation time and extract concentration. Similarly, the study on high-intensity microwave radiation (e.g., 1550 W, 2450 MHz) reported complete cyst mortality within minutes due to substantial temperature increases (29). However, this study found lower efficacy for both microwave treatments and tea extracts compared to gentamicin. This discrepancy may result from differences in microwave intensity, exposure duration, or tea extract concentrations. The reduced mortality rate with tea extract compared to gentamicin may also reflect variations in the type of drug or extract concentrations used.

Recommendation:

-Evaluate the effects of higher power settings, longer exposure times, and varying frequencies to determine the most effective microwave treatment conditions.

-Investigate other plant-derived extracts with potential anti-*Acanthamoeba* properties, focusing on concentration, incubation time, and their interaction with microwave radiation.

-Test combination therapies on diverse *Acanthamoeba* strains and under different environmental conditions to enhance generalizability.

- Assess the feasibility, safety, and cost-effectiveness of integrating microwave radiation with conventional or botanical therapies in clinical and field settings.

CONCLUSION

The integration of microwave radiation with gentamicin or tea extract shows promise as a combination therapy against *Acanthamoeba* cysts. However, the inconsistent results observed in this study highlight the complexity of synergistic interactions and the need for further comprehensive studies to confirm and refine these findings.

***Ethics**

Ethics Committee Approval: All experiments was approved by Ethical Committee of the Arak University of Medical Sciences in this research (number: REC.1400.198, date: 2021-10-31).

Informed Consent: This study was conducted exclusively on *Acanthamoeba* cysts *in vitro*. No human participants or animal subjects were involved; therefore, ethical approval and informed consent were not required.

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Footnotes

***Authorship Contributions**

Surgical and Medical Practices: H.S., R.H., Y.F., Concept: Z.E., H.S., Design: Z.E., H.S., Data Collection or Processing: Z.E., H.S., Analysis or Interpretation: Z.E., H.S., Literature Search: Z.E., H.S., Writing: Z.E., H.S.

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REFERENCES

1. Javanmard E, Kazemirad E, Rahimi HM, Mohebali M, Rezaeian M, Alimohammadi M, et al. Prevalence of free-living amoebae in five rivers associated with high human activity in Tehran province, Iran. J Water Health. 2025; 23: 493-506.
2. Pezeshki A, Tajic S, Farahmandian P, Haniloo A, Mahmmodzadeh A, Niyyati M, et al. Phylogenetic analysis of *Acanthamoeba* isolated from soil samples and nasal cavities of patients with malignancy: a public health concern in the northwest of Iran. J Parasit Dis. 2024; 118: 367-75.
3. Szentmáry N, Daas L, Shi L, Laurik KL, Lepper S, Milioti G, et al. *Acanthamoeba* keratitis–clinical signs, differential diagnosis and treatment. Ophthalmol Ther. 2019; 31: 16-23.
4. Rezeaian M, Farnia S, Niyyati M, Rahimi F. Amoebic keratitis in Iran (1997-2007). Iran J Parasitol. 2007; 2: 1-6.
5. Niyyati M, Rezaeian M. Current status of *Acanthamoeba* in Iran: a narrative review article. Iran J Ophthalmol Parasitol. 2015; 10: 157.
6. Seal DJ. Treatment of *Acanthamoeba* keratitis. Eye Res Ophthalmol-Int. 2003; 1: 205-8.
7. Bisen AC, Sanap SN, Agrawal S, Biswas A, Mishra A, Verma SK, et al. Etiopathology, epidemiology, diagnosis, and treatment of fungal keratitis. ACS Infect Dis. 2024; 10: 2356-80.
8. Karunarathna I, Gunasena P, Gunathilake S, De Alvis K. The clinical use of gentamicin: Indications, mechanism of action, and key considerations. ResGate. Available from: https://www.researchgate.net/publication/383220508; 2024.
9. Hajihossein R, Eslamirad Z, Rafiei F, Naderi G, Assadi M. Anti-acanthamoeba effect of camellia sinensis extract (black and green tea) *in vitro*. J Med Pharm Sci. 2020; 19: 163-9.
10. Kahraman M, PZ. Are thermotolerant and osmotolerant characteristics of *Acanthamoeba* species an indicator of pathogenicity? Turkiye Parazitol Derg. 2024; 48: 15-20.
11. Naderi NJ, Niakan M, Kharazi Fard MJ, Zardi S. Antibacterial activity of Iranian green and black tea on *Streptococcus mutans*: an *in vitro* study. J Dent Tehran. 2011; 8: 55-9.
12. Cabrera C, Artacho R, Giménez R. Beneficial effects of green tea--a review. J Am Coll Nutr. 2006; 25: 79-99.
13. Wang C, Han J, Pu Y, Wang X. Tea (Camellia sinensis): a review of nutritional composition, potential applications, and omics research. Appl Sci. 2022; 12: 5874.
14. Abdolmaleki F. Chemical analysis and characteristics of black tea produced in north of Iran. J Food Biosci Technol. 2016; 6: 23-32.
15. Yang CS, Wang H, Sheridan ZP. Studies on prevention of obesity, metabolic syndrome, diabetes, cardiovascular diseases and cancer by tea. J Food Drug Anal. 2018; 26: 1-13.
16. Teixeira AM, Sousa C. A review on the biological activity of camellia species. Molecules. 2021; 26.
17. Almajano MP, Carbó R, Jiménez JAL, Gordon MH. Antioxidant and antimicrobial activities of tea infusions. Food Chem. 2008; 108: 55-63.
18. Alkufeidy RM, Ameer Altuwijri L, Aldosari NS, Alsakabi N, Dawoud TM. Antimicrobial and synergistic properties of green tea catechins against microbial pathogens. J King Saud Univ Sci. 2024; 36: 103277.
19. Sharangi A. Medicinal and therapeutic potentialities of tea (Camellia sinensis L.)–a review. Food Res Int. 2009; 42: 529-35.
20. Leung HKM, Lo EKK, Zhang F, Felicianna, Ismaiah MJ, Chen C, et al. Modulation of Gut microbial biomarkers and metabolites in cancer management by tea compounds. Int J Mol Sci. 2024; 25: 6348.
21. Botsa E, Thanou I, Nikas I, Thanos L. Treatment of hepatic hydatid cyst in a 7-year-old boy using a new type of radiofrequency ablation electrode. Am J Case Rep. 2017; 18: 953-8.
22. Vecsei Z, Knakker B, Juhász P, Thuróczy G, Trunk A, Hernádi I. Short-term radiofrequency exposure from new generation mobile phones reduces EEG alpha power with no effects on cognitive performance. Sci Rep. 2018; 8: 18010.
23. Zhang Z, Wang J, Hu Y, Wang L. Microwaves, a potential treatment for bacteria: a review. Front Microbiol. 2022; 13: 888266.
24. Eslamirad Z, Soleimani H. Review of non-ionized electromagnetic waves effects on human parasites: a systematic review. J Liaquat Univ Med Health Sci. 2024; 23: 1-10.
25. Zhao X, Dong G, Wang C. The non-thermal biological effects and mechanisms of microwave exposure. J Radiat Res Radiat Oncol. 2021; 19: 483-94.
26. Eslamirad Z, Soleimani H. Investigating the potential of protoscolices for cyst formation under *in vivo* microwave radiation. Complementary Med J. 2019; 9: 3598-606.
27. Eslamirad Z, Hajihossein R, Soleimani H. Is shortwave diathermy effective on mortality of protoscolices? Open Access Maced J Med Sci. 2020; 8: 55-8.
28. Eslamirad Z, Haji Hajihossein R, Soleimani H. Evaluating potential of electromagnetic microwaves on Destruction of *Acanthamoeba* cysts. Complementary Med J. 2020; 9: 3868-77.
29. Eslamirad Z, Soleimani H, Hajihossein R, Rafiei F. Evaluation of lethal effect of microwave exposure on protoscolices of hydatid cyst *in vitro*. J Parasitol Trop Dis. 2015; 5: 821-4.

30. Soleimani H, Hajihossein R, Eslamirad Z. Mobile phone radiation: its efficacy as protoscolicidals. J Trop Parasitol Dis. 2020; 44: 203-6.

31. Meighani M, Eslamirad Z, Hajihossein R, Ahmadi A, Saki S. Isolation and genotyping of *Acanthamoeba* from soil samples in Markazi Province, Iran. Open Access Maced J Med Sci. 2018; 6: 2290-4.

32. Mahmoudi MR, Kazemi B, Haghighi A, Karanis P. Detection of Acanthamoeba and Toxoplasma in river water samples by molecular methods in Iran. Iran J Parasitol. 2015; 10: 250-7.

33. Degerli S, Tepe B, Celiksoz A, Berk S, Malatyali E. *In vitro* amoebicidal activity of Origanum syriacum and Origanum laevigatum on *Acanthamoeba castellanii* cysts and trophozoites. Exp Parasitol. 2012; 131: 20-4.

34. Ghadesi A, Hasanpour H, Naserifar R, Abdi J, Mahmoudi MR, Turki H, et al. Surface water contamination with *Acanthamoeba* spp. in Ilam city, Iran. J Water Sanit Hyg Dev. 2024; 14: 302-12.

35. Thongseesuksai T, Wongwai P, Boonmars T, Sanpool O, Laummaunwai P. Evaluating the in vitro efficacy of gatifloxacin, levofloxacin and gentamicin against *Acanthamoeba* cysts. Int Ophthalmol. 2020; 40: 361-8.

36. Kamiloglu S, Sari G, Ozdal T, Capanoglu E. Guidelines for cell viability assays. Food Front. 2020; 1: 332-49.

37. Mitsuwan W, Sin C, Keo S, Sangkanu S, de Lourdes Pereira M, Jimoh TO, et al. Potential anti-Acanthamoeba and anti-adhesion activities of *Annona muricata* and *Combretum trifoliatum* extracts and their synergistic effects in combination with chlorhexidine against *Acanthamoeba triangularis* trophozoites and cysts. Heliyon. 2021; 7: e06976.

38. Shaw P, Kumar N, Mumtaz S, Lim JS, Jang JH, Kim D, et al. Evaluation of non-thermal effect of microwave radiation and its mode of action in bacterial cell inactivation. Sci Rep. 2021; 11: 14003.

39. Sazgarnia A, Taheri AR, Soudmand S, Parizi AJ, Rajabi O, Darbandi MS. Antiparasitic effects of gold nanoparticles with microwave radiation on promastigotes and amastigotes of Leishmania major. Int J Hyperthermia. 2013; 29: 79-86.

40. Jin Y, Liang X, An Y, Dai Z. Microwave-triggered smart drug release from liposomes co-encapsulating doxorubicin and salt for local combined hyperthermia and chemotherapy of cancer. Bioconjug Chem. 2016; 27: 2931-42.

41. Chegeni TN, Fakhar M, Ghaffarifar F, Saberi R. Medicinal plants with anti-*Acanthamoeba* activity: a systematic review. Infect Dis Ther. 2020; 20: 620-50.

42. Chen C, Chen Y, Wu P, Chen B. Update on new medicinal applications of gentamicin: evidence-based review. J Tradit F Med Arom. 2014; 113: 72-82.

43. López-Salazar H, Camacho-Díaz B, Ocampo M, Jiménez-Aparicio A. Microwave-assisted extraction of functional compounds from plants: a review. BioResources. 2023; 18.

44. Cheng T, Torres NS, Chen P, Srinivasan A, Cardona S, Lee GC, et al. A facile high-throughput model of surface-independent *Staphylococcus aureus* biofilms by spontaneous aggregation. mSphere. 2021; 6: e00186-21.