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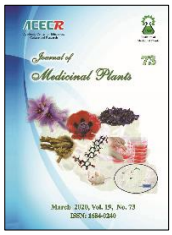

Research Article

Anti-*Acanthamoeba* effect of *Camellia sinensis* extract (black and green tea) *in vitro*

Reza Hajihossein[1], Zahra Eslamirad[1,*], Fatemeh Rafiei[2], Gholamreza Naderi[3], Morteza Assadi[4]

[1]*Department of Parasitology and Mycology, School of Medicine, Arak University of Medical Sciences, Arak, Iran*
[2]*Department of Biostatistics, School of Medicine, Arak University of Medical Sciences, Arak, Iran*
[3]*Department of Agriculture and Natural Resources, Islamic Azad University, Arak Branch, Arak, Iran*
[4]*Department of Biochemistry, Islamic Azad University, Tehran Branch, Tehran, Iran*

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ABSTRACT

Background: *Acanthamoeba* is a resistant protozoan that causes severe diseases, such as GAE and CAK. Because many medications are ineffective on the parasite, the quest to find alternative drugs is in progress. **Objective:** This research was aimed to assess the performance of the black and green tea extracts on *Acanthamoeba*. **Methods:** The clinical isolate of *Acanthamoeba* was cultured on non-nutrient agar plates. The black and green tea extracts were prepared via maceration, dried by rotary evaporation, and stored at 4 °C. The main component of the extracts (caffeine) was recognized using GC-MS analysis. The effects of three concentrations of black and green tea extracts were evaluated on the parasite and compared with the control and current drug. **Results:** Black and green tea extracts have lethal effects on *Acanthamoeba* cysts and the performance was more than that shown by the control and the current anti-keratitis drug. Moreover, the effectiveness of the tea extracts was time- and dose-dependent ($P < 0.0001$). There was no significant differences between the performance of black and green tea ($P > 0.05$). **Conclusion:** Black and green tea extracts demonstrated the potential to inhibit the *Acanthamoeba* parasite, but the use of tea extract in clinical applications requires further study.

1. Introduction

Acanthamoebiasis is a parasitic disease resulted from the amoebic protozoan *Acanthamoeba*. This microorganism is extremely resistant and can be separated from soil and water sources [1].

Due to the widespread distribution of this parasite, many people are exposed to it; however, colonization only occurs in immunocompromised patients. Granulomatous amoebic encephalitis

Abbreviations: GAE, Granulomatous Amoebic Encephalitis; CAK, Chronic Amoebic Keratitis; NNA, Non Nutrient Agar; SKUMS, Shahrekord University of Medical Scences; GC-MS, Gas Chromatography-Mass Spectrometry

* Corresponding author: dr.eslami@arakmu.ac.ir

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(GAE) and chronic amoebic keratitis (CAK) are the most severe types of the disease [2-4].

Transition of the disease is carried out by cysts that are resistant to ecological factors [1]. In recent years, compounds such as biguanides and chlorhexidine have been used against trophozoites and cysts of parasite. Biguanides have a toxic effect on human corneal cells. Chlorhexidine is not toxic effect on corneal cell and often used in combination with propamidine, hexamidine and neomycin. But unfortunately, all countries do not have access to propamidine and hexamidine [5].

As the medicines currently utilized for treating amoebic encephalitis and keratitis are ineffective, the search for appropriate remedies continues [6].

Camellia sinensis, which originated from China, is now cultivated over the world. The plant arrived in Iran in 1882 and cultivation started in the Gilan province. After water, the brew of *C. sinensis* leaves (otherwise known as tea) is one of the most common drinks in the worldwide [7]. Green tea refers to a product prepared from the fresh leaves of *C. sinensis* without oxidation, whereas black tea refers to the product that is prepared after the fermentation and oxidation of leaves [8].

In some countries, this plant has been utilized to treat certain illnesses or as a disinfectant. Due to the fact that the antimicrobial properties of some herbal compounds have been clarified, activity of some medicinal plants i.e. *Thymus*, *Satureja*, *Melissa*, *Origanum*, *Helianthemum*, *Arachis* against trophozoite and cyst of Acanthamoeba were studied. The results of most of these studies indicate that the compounds of these plants destroy trophozoites faster than cysts and also require more concentrations of plant compounds to eliminate cysts [9].

In traditional Iranian medicine, tea is used for eye irrigation and to treat inflammatory ocular infections; thus, this research was conducted to study the impacts of green and black tea on *Acanthamoeba* in vitro and to compare the effects with Natamycin, a routine drug utilized for treating keratitis.

2. Materials and Methods

2.1. Parasites

A clinical isolate of *Acanthamoeba* spp. was prepared by a clinician. The samples were cultured on non-nutrient agar plates (NNA) coated with killed *Escherichia coli* at temperature of 28 °C. After 1-3 weeks, the surface of plate was washed with sterile Page's saline and the cysts were collected and concentrated via centrifuging at 1500 × *g* for 5 min [10]. A suspension that contained 10^5 cysts/ml were utilized for all experiments.

2.2. Plant extraction

C. sinensis, as green and black tea, was attained from Medical Plants Research Center, SKUMS, and transferred to the laboratory of Infectious Diseases Research Center in School of Medicine, Arak University of Medical Sciences. The extracts of the green and black tea were prepared via maceration as follows: 100 g tea (green and black, separately) was transferred into an Erlenmeyer flask, 1 L of 70% ethanol was added and the solution was put at the laboratory temperature for 24 h. The extract was filtered through filter paper, dried by rotary evaporation, and stored at 4 °C [11].

2.3. Gas Chromatography-Mass Spectrometry (GC-MS) analysis

GC-MS analysis was performed using a Perkin Elmer Clarus 680 (GC) and a Perkin Elmer Clarus SQ 8t (Shelton. CT 06484, USA)

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(MS) equipped with a silica capillary Elite 5 MS column (30 m × 0.25 mm i.d.; film thickness, 0.25 µm). Helium was utilized as the carrier gas with ionization voltage of 70 eV. The interface temperature was 250 °C and the mass range considered was from 40-500 (m/z). The oven temperature was increased from 30 °C to 300 °C at a rate of 10 °C/min and maintained at 300 °C for 15 min. The injector temperature was 220 °C and the injector volume was 0.2 µl. The total run time was 41 min.

2.4. Experiment

All tests were carried out in 1.5-ml micro tubes. The samples were divided into ten groups, which were treated with different materials: PBS + DMSO (the negative control), formalin (the positive control), the alcohol control, 0.0025 µg/ml Natamycin (common test drug), black tea at 80, 160, and 320 mg/ml, and green tea at 80, 160, and 320 mg/ml. Each group was assessed over eight different time periods (0, 10, 20, 30, 60, 120 min, 24 h, and 48 h) in different micro tubes. All micro tubes contained 200 µl of parasite suspension.

The viability of the cysts was tested via the trypan blue method and the mortality rate was measured by counting cells with a hemocytometer. The stained cysts were considered as dead (Fig. 1B), whereas the unstained cysts were recorded as alive (Fig. 1A) [12].

2.5. Data analysis

Statistical analyses were conducted using SPSS software version 13 and MedCalc software version 7. The comparison of the mortality between groups was evaluated via two-tail analysis variation tests and $P<0.05$ was taken into account statistically significant. The charts were prepared using MedCalc software.

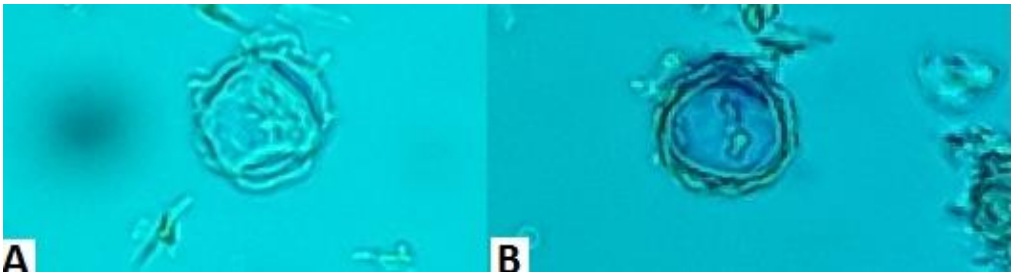

Fig. 1. *Acanthamoeba* cyst. (A) Living cysts. (B) Dead cysts.

3. Results

Caffeine was the main component of the two ethanolic extracts, with 88 % and 75 % in black tea and green tea extracts, respectively.

The outcomes indicated that the black and green tea extracts exerted lethal effects on *Acanthamoeba* cysts. The effects were stronger than that exhibited by PBS + DMSO (the negative control), the alcohol control, and Natamycin. Additionally, the onset of the lethal effect of tea (black and green) was more rapid than that of the Natamycin (Fig. 2).

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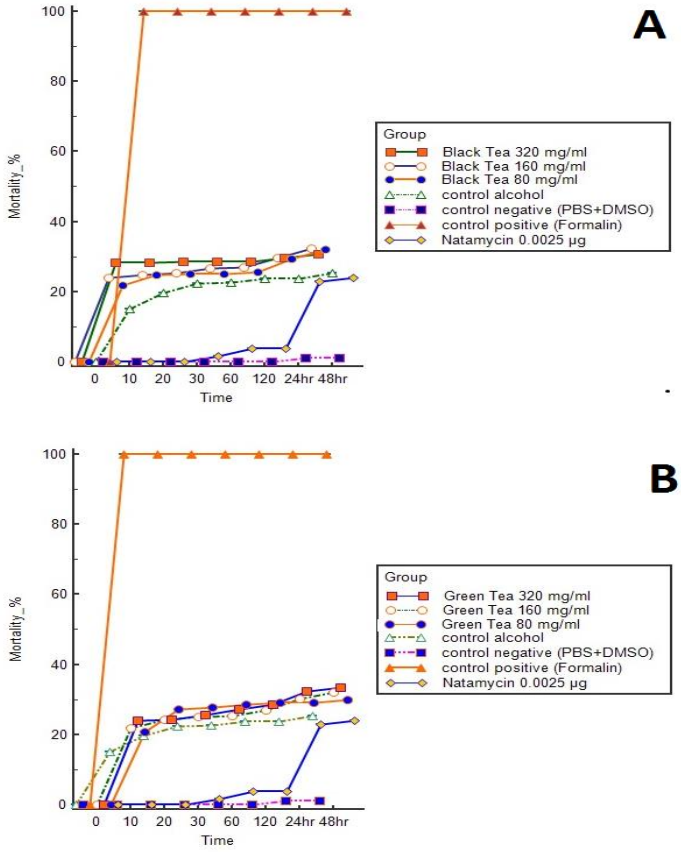

Fig. 2. A comparison of the mortality rate of *Acanthamoeba* cysts treated with (A) black tea and (B) green tea along with negative control, positive control, alcohol control, and Natamycin

The statistical analysis indicated that the effects of both black and green tea were time- ($P<0.01$) and dose-dependent ($P<0.0001$). An increase in the mortality rate of *Acanthamoeba* during the exposure time of three different concentrations of green and black tea was steady and slow (Fig. 2). However, the mortality rate of the parasite in the first 2 h of exposure to the Natamycin was very low and then significantly increased. Significant difference was not found in the lethality of black and green tea ($P>0.05$).

4. Discussion

Acanthamoeba is an opportunistic free amoeba and one of the causes of keratitis. Although the occurrence of amoebic keratitis is low compared with that of other types of keratitis (viral, fungal, and bacterial keratitis), it has recently increased owing to risk factors like the usage of contact lenses [13]. The prevalence of *Acanthamoeba* keratitis because of the contact of the eyes with polluted water has been proven. As the pollution of soil sources with a pathogenic genotype of *Acanthamoeba* was confirmed, it seems that airborne fine dust can have a role in the transmission of parasitic cysts [14, 15]. However, further study is needed to prove this.

In this in vitro study, the performance of black and green tea on *Acanthamoeba* cysts were compared with the efficacy of three different control solutions and a common drug used for keratitis treatment. In current research, the anti-*Acanthamoeba* property of black and green tea was related to the time of exposure and the

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applied extract dose. The anti-*Acanthamoeba* activity of Natamycin, a common treatment of keratitis, was effective from 30 min following exposure to the parasite, but the activity of black and green tea was effective from 10 min after exposure. In addition, the mean of the death in parasites exposed to different doses of black and green tea was higher than the negative control (PBS + DMSO), the alcohol control, and Natamycin.

Recently, the impact of tea on health has been given importance. The application of the aqueous extract of *C. japonica* petals exhibited an inhibitory impact on the growth of some foodborne pathogens in culture media. However, significant difference was not observed in the inhibitory effect between the extract and the control [16]. In our study, the mortality rate of the parasites increased following exposure to tea extracts. Additionally, the anti-*Acanthamoeba* activity of black and green tea was considerably more than that of the control and Natamycin. This is interesting; as the culture medium of *Acanthamoeba* is including a liquid phase, using the cultivation method is impossible for evaluating the impact of the plants components on the parasite growth.

Many investigations have reported contradictory effects of tea on microorganisms. As an example, in one study, the antibacterial activities of several kinds of tea were compared and the results showed that black tea had no antibacterial activity [17]. In contrast, another research indicated that the extract of six types of tea (including green and black tea) controlled the growth of gram-positive bacteria [18].

In a study of the impact of black tea on conjunctivitis, the results showed that 100 mg/ml black tea extract had a more inhibitory activity than that of certain antibiotics. Additionally, these effects were time- and dose-dependent [19]. The target microorganisms in our study were different to this study, but the outcomes were almost identical; the only difference was that at the end of the exposure period, the mortality rate for the parasite exposed to 160 mg/mL black tea was more than that exposed to 320 mg/ml.

In another study, the effectiveness of different concentrations of green tea on *Enterococcus faecalis* was compared with that of 1% sodium hypochlorite. In contrast with our results, this study indicated that the effectiveness of green tea aqueous extract was considerably lesser than that of 1% sodium hypochlorite [20].

5. Conclusion

In recent years, it has been reported that many of the plant compounds have anti-*Acanthamoeba* properties, so that these compounds can be considered for the purpose of searching for new anti- *Acanthamoeba* compounds. Tea extract can inhibit or kill disease-causing microorganisms. Thus, it may be effective for treating some infections, but more in-depth studies are needed to prove this behavior.

Author contributions

Reza Hajihossein participated in preparation of parasite and conduct of experiments; Zahra Eslamirad participated in drafting proposals, conduct of experiments, prepare of manuscript; Fatemeh Rafiei participated in Data analysis; Gholamreza Naderi participated in plant extraction; Morteza Assadi participated in determining components of green tea and black.

Conflict of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

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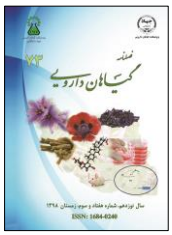

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مقاله تحقیقاتی

اثر ضد آکانتامبایی عصاره چای سیاه و چای سبز در شرایط برون‌تنی

رضا حاجی حسین[۱]، زهرا اسلامی‌راد[۱،*]، فاطمه رفیعی[۲]، غلامرضا نادری[۳]، مرتضی اسدی[۴]

[۱] گروه انگل‌شناسی و قارچ‌شناسی، دانشکده پزشکی، دانشگاه علوم پزشکی اراک، اراک، ایران

[۲] گروه آمار زیستی، دانشکده پزشکی، دانشگاه علوم پزشکی اراک، اراک، ایران

[۳] گروه کشاورزی و منابع طبیعی، دانشگاه آزاد اسلامی، واحد اراک، اراک، ایران

[۴] گروه بیوشیمی، دانشگاه آزاد اسلامی، واحد تهران، تهران، ایران

اطلاعات مقاله

گل‌واژگان:
آکانتامبا
چای سیاه
چای سبز
درمان مکمل
طب سنتی

چکیده

مقدمه: آکانتامبا تک یاخته‌ای مقاوم است که باعث بیماری‌های شدید مانند انسفالیت گرانولوماتوز آمیبی و کراتیت مزمن آمیبی می‌شود. از آنجا که بسیاری از داروها روی انگل بی‌اثر هستند، تلاش برای یافتن داروهای جایگزین در حال انجام است. **هدف:** این تحقیق با هدف ارزیابی عملکرد عصاره چای سیاه و سبز بر روی آکانتامبا انجام شد. **روش بررسی:** ایزوله بالینی آکانتامبا در پلیت‌های آگار غیرمغذی کشت داده شد. عصاره چای سیاه و سبز از طریق ماسراسیون تهیه شد و به روش تقطیر در خلاء، خشک شد و در ۴ درجه سانتی‌گراد نگهداری شد. ترکیب اصلی عصاره (کافئین) با استفاده از کروماتوگرافی گازی متصل به طیف‌سنج جرمی شناسایی شد. تأثیر سه غلظت عصاره چای روی انگل مورد ارزیابی قرار گرفت و با گروه کنترل مقایسه شد. **نتایج:** عصاره‌های چای سیاه و سبز دارای اثرات کشنده‌ای بر روی کیست‌های آکانتامبا بوده و اثر آن بیشتر از داروی ضد کراتیت موجود است (گروه کنترل). علاوه بر این، اثر عصاره چای وابسته به زمان و دوز است ($P < ۰/۰۰۰۱$). همچنین بین عملکرد چای سیاه و سبز تفاوت معنی‌داری مشاهده نشد. **نتیجه‌گیری:** عصاره‌های چای سیاه و سبز پتانسیل مهار انگل آکانتامبا را دارند، اما استفاده از عصاره چای در کاربردهای بالینی نیاز به مطالعه بیشتر دارد.

مخففها: GAE، انسفالیت گرانولوماتوزآمیبی؛ CAK، کراتیت آمیبی مزمن؛ NNA، آگار غیرمغذی؛ SKUMS، دانشگاه علوم پزشکی شهرکرد؛ GC-MS، کروماتوگرافی گازی متصل به طیف‌سنج جرمی

* نویسنده مسؤول: dr.eslami@arakmu.ac.ir

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