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Evaluation of Antileishmanial Activities of a Peganum harmala and Achillea millefolium Essential Oils and Their Combinations Against Leishmania infantum promastigotes

Peganum harmala ve Achillea millefolium Uçucu Yağlarının ve Kombinasyonlarının Leishmania infantum promastigotes'e Karşı Antileishmanial Aktivitelerinin Değerlendirilmesi

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Abstract

Medicinal plants and their derivations are used as safe agents for the treatment of parasitic diseases. This preliminary study investigates antileishmanial activities of *Peganum harmala* essential oil (PHEO), *Achillea millefolium* essential oil (AMEO) and their combinations against *Leishmania infantum* (*L. infantum*) promastigotes. A standard strain of *L. infantum* promastigote was cultured in a 96-well Novy-MacNeal-Nicolle (NNN) media culture and antileishmanial activities of glucantime, PHEO, AMEO, an equal ratio of both and 80% PHEO+20%AMEO were investigated in concentrations of 10, 100, 500 and 1000 mg/mL and interval times of 24h, 48h and 72h. The results showed that greatest inhibition was observed in 50% PHEO + AMEO and lowest inhibition was seen in control group. The increased time and increased concentration significantly increased their efficiencies. The analyses showed a significant interaction between time and agents [F (10, 360)=7.84, P=0.000]. The agents showed better effects with increased time. In sum, an equal combination of PHEO and AMEO showed its potential as an antileishmanial safe structure and must be considered for future studies.

Keywords: *Achillea millefolium*, Antileishmanial activity, *Peganum harmala*, Promastigote

Özet

Tıbbi bitkiler ve türevleri paraziter hastalıkların tedavisinde güvenli ajanlar olarak kullanılmaktadır. Bu ön çalışma, *Peganum harmala* uçucu yağı (PHEO), *Achillea millefolium* uçucu yağı (AMEO) ve bunların kombinasyonlarının *Leishmania infantum* (*L. infantum*) promastigotlarına karşı antileishmanial aktivitelerini araştırmaktadır. Standart bir *L. infantum* promastigot suşu, 96 kuyulu bir Novy-MacNeal-Nicolle (NNN) kültür ortamında yetiştirildi ve 10, 100, 500 ve 1000 mg/mL konsantrasyonlarda ve 24 saat, 48 saat ve 72 saat aralık sürelerinde glucantime, PHEO, AMEO, ikisinin eşit oranında ve %80 PHEO+%20 AMEO'nun antileishmanial aktiviteleri araştırıldı. Sonuçlar, en yüksek inhibisyonun %50 PHEO + AMEO'da gözlemlendiğini ve en düşük inhibisyonun kontrol grubunda görüldüğünü gösterdi. Artan zaman ve artan konsantrasyon, verimliliklerini önemli ölçüde artırdı. Analizler, zaman ve ajanlar arasında önemli bir etkileşim olduğunu gösterdi [F (10, 360)=7.84, P=0.000]. Ajanlar, artan süre ile daha iyi etkiler gösterdi. Özetle, PHEO ve AMEO'nun eşit kombinasyonu, antileishmanial güvenli bir yapı olarak potansiyelini göstermiştir ve gelecekteki çalışmalar için dikkate alınmalıdır.

Anahtar Kelimeler: *Achillea millefolium*, Antileishmanial aktivite, *Peganum harmala*, Promastigot

Abbreviations: AMEO, *Achillea millefolium* essential oil; MTT, Methyl thiazole tetrazolium; PHEO, *Peganum harmala* essential oil; NNN, Novy-MacNeal-Nicolle

1. INTRODUCTION

Leishmania infantum is one of the Leishmania species that causes visceral leishmaniasisis (Zheng et al., 2020). The disease is caused by parasitic protozoan and transmitted by the bites of infected female phlebotomine sandflies (Cabral et al., 2020). The protozoa cause serious challenges in all over the world and more than one billion people are at risk for the disease (mondiale de la Santé & Organization, 2021). It causes clinical signs from cutaneous form to the visceral form, fever, splenomegaly (enlargement of the spleen, manifested in the great majority of patients), hepatomegaly (enlargement of liver), pallor (caused by severe anemia), leucopenia (low white blood cell count), and weight loss (Gervazoni et al., 2020). Leishmania has two stages in its life cycle, including promastigote, and amastigote (Tavakoli et al., 2020). Promastigote is developed in sand fly body while amastigote is formed in macrophage (De Queiroz et al., 2014). Various agents are utilized to treat leishmaniasis. Glucantime has traditionally been used for the treatment of leishmaniasis (Lima et al., 2010). The current antileishmanial agents have limitations such as side effects, prolonged treatment period, high costs and induction of parasitic resistance (Herrera et al., 2020). Since antileishmanial drugs have limitations, researchers have sought novel drugs. Herbal medicine and their derivations

such as herbal extracts and essential oils have used as antileishmanial agents (Ayrom et al., 2021; de Paula et al., 2019; Delgado-Altamirano et al., 2017).

Esfand (*Peganum harmala* L.,) belongs to the family Zygophyllaceae and is found in Mediterranean regions such as Iran and Turkey (Asadzadeh et al., 2021). It contains a huge amount of seed (Asgarpanah & Ramezanloo, 2012) β-carboline alkaloids, quinazoline alkaloids, steroids, anthraquinones, flavonoids, and amino acids (Shao et al., 2013). Several studies have reported pharmaceutical properties of *P. harmala* essential oil such antimicrobial activities (Apostolico et al., 2016; Hajji et al., 2020; Khadhr et al., 2017). Studies have reported antileishmanial activity of *P. harmala* against *L. major* (Rahimi-Moghaddam et al., 2011).

Yarrow plant (*Achillea millefolium*) belongs the Asteraceae family and it is found in Asia, European and America (Acar et al., 2020). It is mainly contained amazulene, α-pinene, β-pinene, casticin, 1,8-cineole,cosmosiin and luteolin (Ali et al., 2017). It is known to have some properties such as anti-inflammatory, antipyretic, anthelmintic, antibacterial, antifungal, antitumor, antioxidant and anti-oedematous (Daniel et al., 2020). Studies have reported antileishmanial activity of *A. millefolium* essential oil (Santos et al., 2010).

Methyl thiazole tetrazolium (MTT) colorimetric methodies used cytotoxicity analyzes to human or animal cells (Ayrom et al., 2021).

A combination of both plants can have better antileishmanial activity in against *Leishmania infantum* promastigote. This preliminary study investigates antileishmanial activities of *P. harmala* essential oil (PHEO), *A. millefolium* essential oil (AMEO) and their combinations against *L. infantum* promastigotes.

2. MATERIALS AND METHODS

2.1. The Preparation of Essential Oils

The aerial parts of the *A. millefolium* and *P. harmala* seeds were prepared from a local market in the West-Azerbaijan province of Iran and identified by an expert botanist in Biology Department in Islamic Azad University, Urmia Branch. A. millefolium was prepared as reported by previous studies (Daniel et al., 2020). Briefly, aerial parts were dried, ground, and extracted by hydro distillation in Clevenger apparatus. *P. harmala* essential oil was prepared by hydro distillation in Clevenger apparatus as reported by previous studies (Yang et al., 2020). The prepared essential oils were dried over sodium sulfate anhydrous and kept at 0ºC after filtration. The aerial parts yielded for *A. millefolium* and *P. harmala* oils were 1.10% and 1.32% dry weight of the plant material, respectively.

2.2. Cultivation of *L. infantum* promastigote

A standard strain of *L. infantum* (MCAN/IR/96/LON49)) promastigote was provided from Urmia University of Medical Science and cultured in a 96-well Novy-Mac Neal-Nicolle (NNN) medium containing antibiotics as reported by previous studies (Ayrom et al., 2021).

2.3. MTT Test

The tests were conducted based on previous studies (Ayrom et al., 2021). Summary, promastigotes were cultured and incubated. MTT material was added it, incubated, removed and loaded with 100 µL DMSO (Toray Fine Chemicals Co., Ltd.). Densities were investigated by ELISA reader (Stat fax 2100, USA) at the wavelength of 570 nm. The most appropriate concentration of promastigote was 106 parasites/ml. Following dilution of promastigotes with liquid media of 1640 RPMI (Thermo Fisher Scientific Company), they were transferred into plates containing media culture and investigated in smear form. Various concentrations (10, 100, 500 and 1000 mg/mL) of PHEO, AMEO, Glucantime, 80%PHEO+20% AMEO (80PHEO+AMEO) and 50%PHEO+50% AMEO (50PHEO+ AMEO) were tested in time intervals of 24, 48 and 72 hours. We also considered wells lack of essential oil and Glucantime as control. Five replications were considered for each treatment in specific time points.

2.4. Data Analysis

The data were analyzed for normality by Kolmogorov-Smirnov test and the data were normal. The data were analyzed in a factorial arrangement with six agents (control, Glucantime, PHEO, AMEO, 80PHEO+AMEO, and 50PHEO+ AMEO), four concentrations (10, 100, 500 and 1000 mg/mL) and three interval times (24, 48 and 72 h). Main effects and interactions were investigated by SPSS software (version of 24). A $p<0.05$ was considered as significant.

3. RESULTS and DISCUSSION

Figure 1 shows the effects of commercial agent of glucantime and essential oils on inhibition percentage. The results showed that greatest inhibition was observed in 50PHEO+AMEO and lowest inhibition was seen in control group. Glucantime and 80PHEO+AMEO showed greatest antileishmanial activity after 50PHEO+AMEO and did not show significant differences (P=0.721). PHEO had better antileishmanial activity compared to AMEO.

An equal ratio of both essential oils had the best activity while 80% PHEO and 20% AMEO had lower effects. Glucantime showed lower activity compared to an equal ratio of both essential oils. The results for antileishmanial activities of AMEO and PHEO are similar to

results reported by previous studies (Rahimi-Moghaddam et al., 2011; Santos et al., 2010). Pharmacological activity of AMEO is attributed to its active compounds including sesquiterpene lactones, azuleneand flavonoids (Benedek et al., 2006). The inhibitory effects of PHEO could be attributed to its compounds such as β-carbolines and quinazoline derivatives (Mirzaie et al., 2007). β-carboline derivatives have antiparasitic activities. It was reported that other compounds of PHEO such as harmaline have in vivo antileishmanial activity (Evans & Croft, 1987). Other studies have reported antileishmanial activity of β-carboline alkaloids such asharmine and harmane (Di Giorgio et al., 2004). It was reported that plant active compound such as harmine and harmaline prevent mono-amino oxidase type A enzyme and cause psychological disorders such as hallucination (McKenna et al., 1984). An equal combination of PHEO and AMEO had better effects compared to single form and 80% combination that might be attributed to synergistic effects of PHEO and AMEO. A combination of PHEO and AMEO had equal and better effects with commercial agent of Glucantime that are parallel with results reported by previous studies (Ayrom et al., 2021).

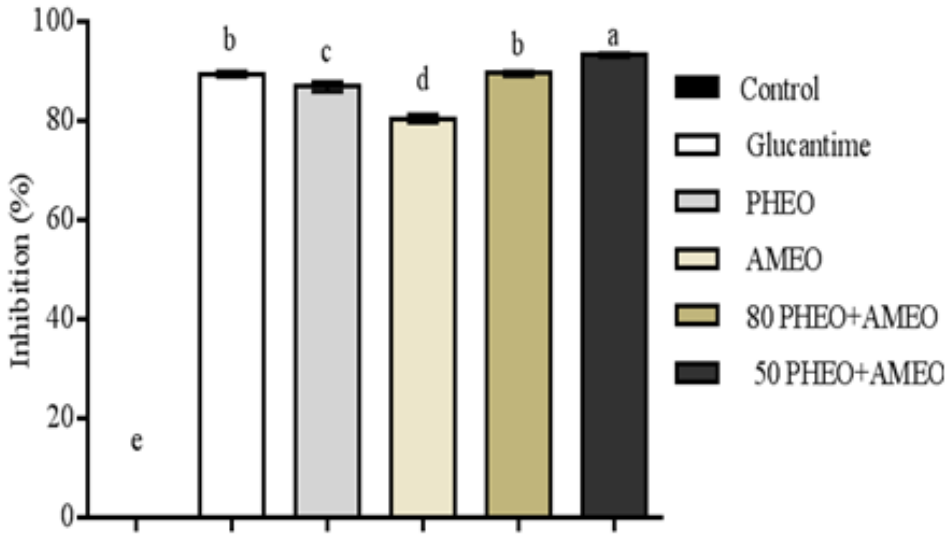

Figure 1. Inhibitory effect of the agents against leishmanial promastigotes. Different letters (a-e) on figures show significant differences between groups.

The results for the effects of different concentrations are shown in Figure 2. The results showed that the lowest antileishmanial activity was observed in concentration of 10 mg/mL and greatest inhibitory effects were seen in concentration of 1000 mg/mL ($P<0.05$). Significant differences were not seen between concentrations of 100 and 500 mg/mL ($P=0.061$). It means that increased concentration increases inhibitory effects. Similar to our findings, previous studies have reported that increased concentration raises antileishmanial activity of essential oils (Ayrom et al., 2021). As mentioned, essential oils show their activities via active

compounds. Having more active compounds causes that essential oils efficiently show their effects. Higher concentrations provide more synergism interaction effects for influencing on Leishmania.

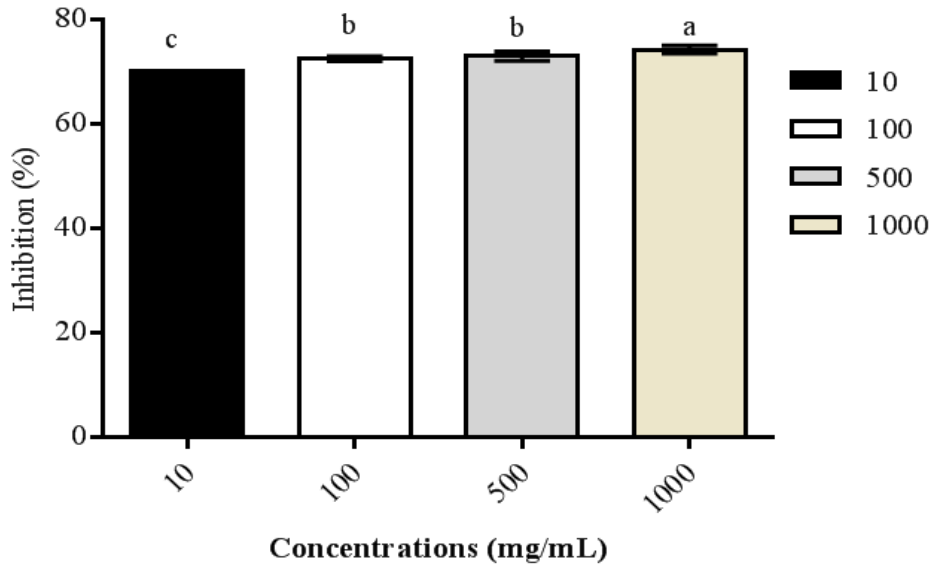

Figure 2. Inhibitory effects of different concentrations against leishmanial promastigotes.

Different letters (a-c) on figures show significant differences between groups.

Figure 3 shows inhibitory effects of treatments in different times against *L. infantum* promastigotes. The results showed that increased time raises inhibitory effects against *L. infantum* promastigotes. The lowest inhibitory effects were seen in time of 24 h while the greatest effects were observed in time of 72 h ($P<0.05$).

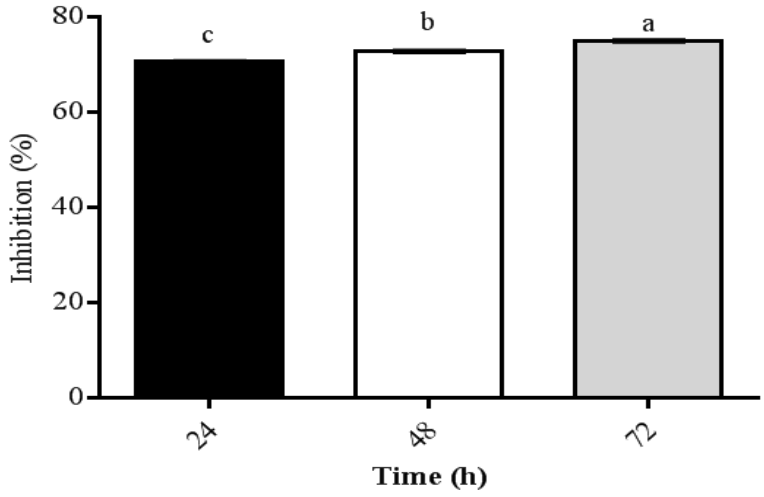

Figure 3. Inhibitory effects of treatments in different times against leishmanial promastigotes.

Different letters (a-c) on figures show significant differences between groups.

The results for the effects of time on inhibition of *L. infantum* are in agreement with previous studies (Ayrom et al., 2021). Seemingly, agents need more time for affecting on parasites and increased time improves its efficiency.

The results for interactions did not show significant differences for interaction between agents and concentration [F (15, 360)=0.836, *P*=0.637], for interaction between time and concentration [F (6, 360)=0.266, *P*=0.952], and also for interaction between agent, time and concentration [F (30, 360)=0.211, *P*=1.00]. The analyses showed a significant interaction between time and agents [F (10, 360) =7.84, *P*=0.000]. The agents showed better effects with increased time (Figure 4).

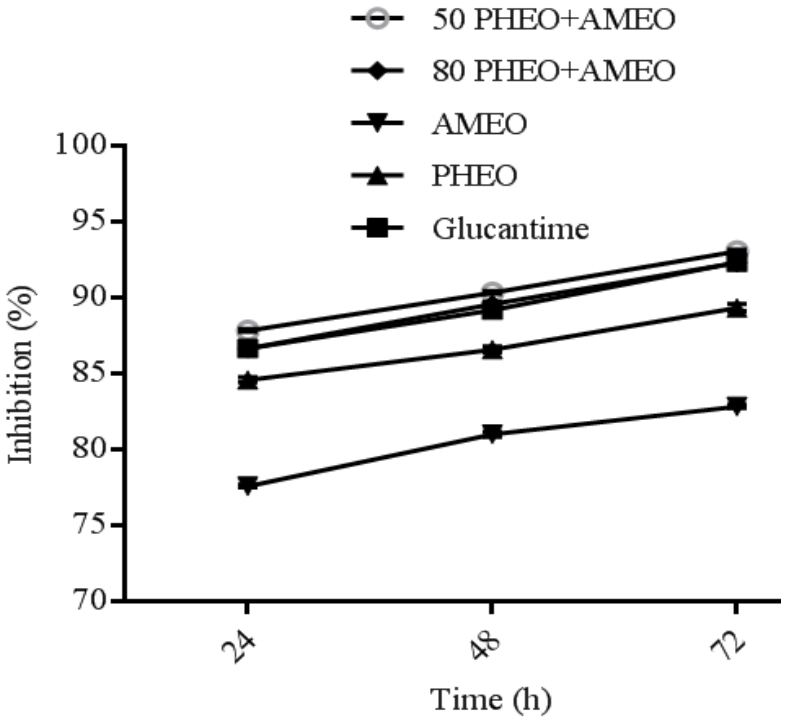

Figure 4. Interaction between treatments in different times of 24, 48 and 72 h.

4. CONCLUSION

In conclusion, a combination of AMEO and PHEO in an equal ratio had the best antileishmanial activity against *L. infantum* promastigotes. An equal ratio of both AMEO and PHEO could compete with synthetic agent of Glucantime and is a safe structure for the treatment of leishmaniasis.

DECLARATIONS

All authors declare that they have no conflicts of interest.

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