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Evaluation of Antileishmanial Activities of a *Peganum harmala* and *Achillea millefolium*: Essential oils and their combinations against Leishmania Infantum Promastigotes

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Research Article

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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 Oils (AMEO) and their combinations against Leishmania infantum promastigotes. A standard strain of L. infantum promastigote was cultured in a 96-well Novy-MacNeal-Nicolle 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 24 h, 48 h and 72 h. The results showed that greatest inhibition was observed in 50PHEO+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.

INTRODUCTION

Visceral leishmaniasisis an infectious disease is caused by Leishmania infantum [1]. The disease is caused by parasitic protozoan and transmitted by sandflies [2]. The protozoa cause serious challenges in all over the world and more than one billion people are at risk for the disease [3]. It causes clinical signs from cutaneous form to the visceral form [4]. Leishmania has two stages in its life cycle, including promastigote, and amastigote [5]. Promastigote is developed in sand fly body while amastigote is formed in macrophage [6]. Various agents are utilized to treat leishmaniasis. Glucantime has traditionally been used for the treatment of leishmaniasis [7]. The current antileishmanial agents have limitations such as side effects, prolonged treatment period, high costs and induction of parasitic resistance [8]. 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 [9-11].

Esfand (*Peganum harmala L*) belongs to the family *Zygophyllaceae* and is found in Mediterranean regions such as Iran and Turkey [12]. It contains a huge amount of seed [13]. It contains β-carboline alkaloids, quinazoline alkaloids, steroids, anthraquinones, flavonoids, and amino acids [14]. Several studies have reported pharmaceutical properties of *P.harmala* essential oil such antimicrobial activities [15-17]. Studies have reported antileishmanial activity of *P.harmala* against L. major [18].

Yarrow plant (*Achillea millefolium*) belongs to the *Asteraceae* family and it is found in Asia, European and America [19]. It is mainly contained amazulene, α-pinene, β-pinene, casticin, 1,8-cineole,cosmosiin and luteolin[20]. It is known to have some properties such as anti-inflammatory, antipyretic, anthelmintic, antibacterial, antifungal, antitumor, antioxidant and anti-oedematous [21]. Studies have reported antileishmanial activity of *A.millefolium* essential oil [22].

Methyl Thiazole Tetrazolium (MTT) colorimetric methodies used for assessing the effects of the agents on leishmaniasis [9].

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.

MATERIALS AND METHODS

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-Azarbayjan 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 [21]. Briefly, aerial parts were dried, ground, and extracted by hydro distillation in Clevenger apparatus. Essential oil was prepared by hydro distillation in Clevenger apparatus as reported by previous studies [23]. The prepared essential oils were dried over sodium sulfate anhydrous and kept at 0°C 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.

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 [9].

MTT test

The tests were conducted based on previous studies. Summary, promastigotes were cultured and incubated. MTT material was added it, incubated, removed and loaded with 100 µL DMSO. 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, 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.

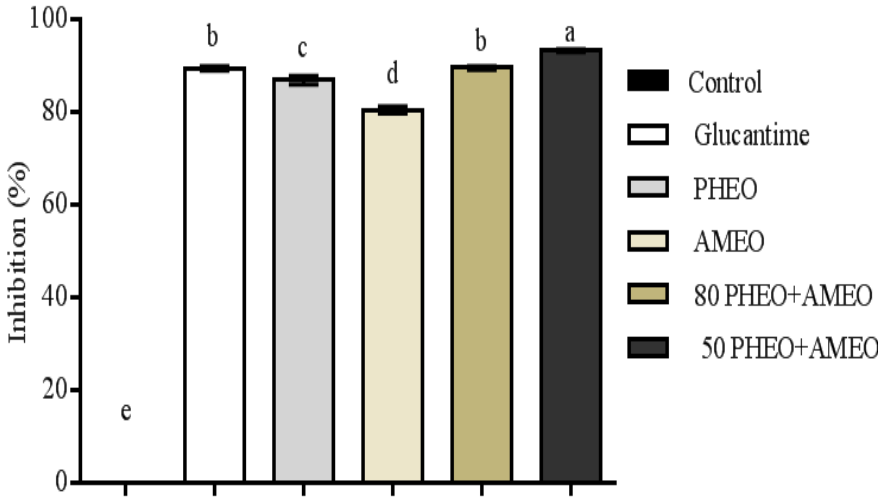
Data analysis

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.

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 80 PHEO+ 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.

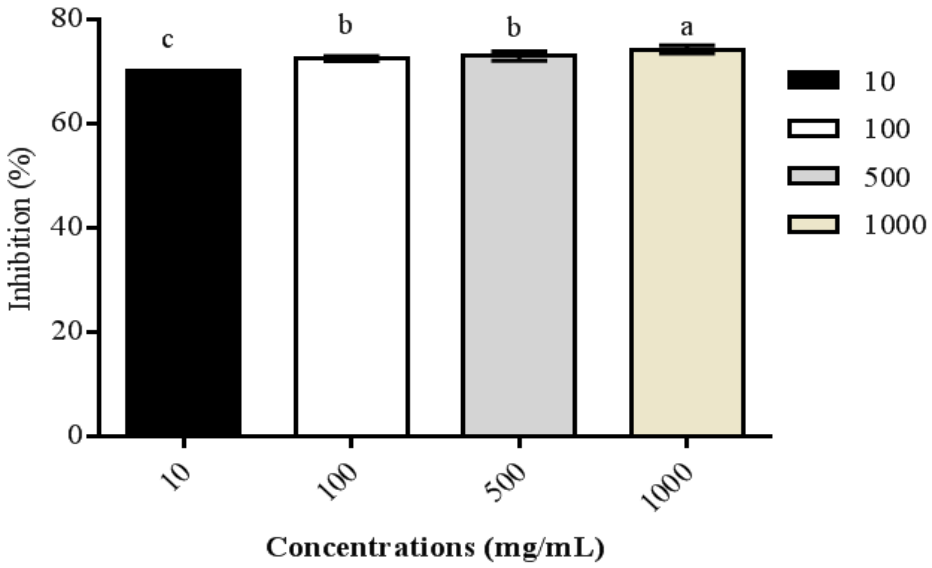
Figure 1. Inhibitory effect of the agents against leishmanial promastigotes. The letters (a-e) show significant differences. ***Note:** a- Control; b- Glucantime; c- PHEO; d- AMEO; b- 80 PHEO+AMEO; a- 50 PHEO+AMEO



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 [18,22]. Pharmacological activity of AMEO is attributed to its active compounds including sesquiterpene lactones, azuleneand flavonoids [24]. The inhibitory effects of PHEO could be attributed to its compounds such as β-carbolines and quinazoline derivatives [25]. β-carboline derivatives have antiparasitic activities. It was reported that other compounds of PHEO such as harmaline have *vivo* antileishmanial activity [26]. Other studies have reported antileishmanial activity of β-carboline alkaloids such asharmine and harmane [27]. 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 [28]. 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 [9].

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. 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 [9]. As mentioned, essential oils show their activities *via* active compounds.

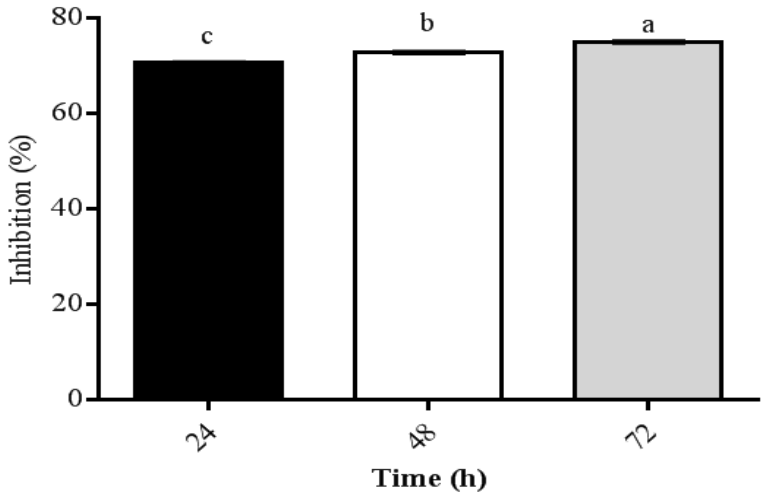
Figure 2. Inhibitory effects of different concentrations against leishmanial promastigotes. The letters (a-c) show significant differences. ***Note:** a- 1000; b- 500; b- 100; c- 10



The results for the effects of time on inhibition of leishmaina are in agreement with previous studies [9]. Seemingly, agents need more time for affecting on parasites and increased time improves its efficiency.

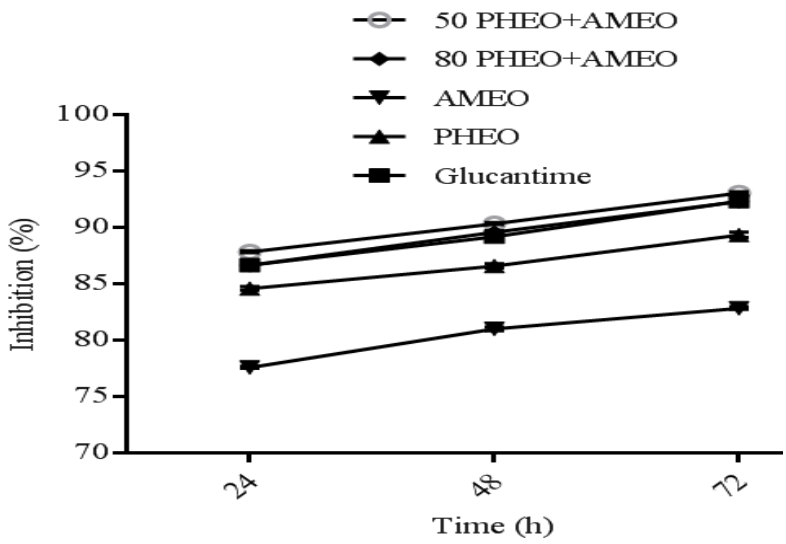
Figure 3 shows inhibitory effects of treatments in different times against leishmanial promastigotes. The results showed that increased time raises inhibitory effects against leishmanial promastigotes. The lowest inhibitory effects were seen in time of 24 h while the greatest effects were observed in time of 72 h.

Figure 3. Inhibitory effects of treatments in different times against leishmanial promastigotes. The letters (a-c) show significant differences. ***Note:** a- 72; b- 48; c- 24



The results for interactions did not show significant interactions between agents and concentration (F (15, 360)=0.836, P=0.637), between time and concentration (F (6, 360)=0.266, P=0.952), and also 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. ***Note:** 50 PHEO+AMEO; 80 PHEO+AMEO; AMEO; PHEO; Glucantime



CONCLUSION

In conclusion, a combination of AMEO and PHEO in an equal ratio had the best antileishmanial activity against L.infantum promastigotes. Having more active compounds causes that essential oils efficiently show their effects. Higher concentrations provide more synergism interaction effects for influencing on leishmania. An equal ratio of both AMEO and PHEO could compete with synthetic agent of Glucantime and is a safe structure for the treatment of leishmania.

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