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Original Article

Evaluating the Anti-Leech Effects of Methanolic Extracts of *Peganum harmala* L. and *Olea europaea* L. on *Limnatis nilotica*

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ABSTRACT

Leeches had several complications such as pain, itching, inflammation, severe anemia, short-term bleeding, hypersensitivity, and anaphylactic reactions in their hosts. Harmal *Peganum harmala* L. is used as an analgesic and anti-inflammatory agent and it has antibacterial activity. Olive *Olea europaea* L. has antibacterial, anti-viral, hypoglycemic and the relaxation of blood vessels properties. Antioxidant properties of olive also had been reported. This study was carried out to detect the effects of methanolic extracts of *P. harmala* L. and *O. europaea* L. on *L. nilotica* immature form. In 2011, 55 immature leeches collected from the southern area of Ilam province were prepared. The methanolic extract of *O. europaea* L and *P. harmala* L. were compared with levamisole as the control drug. Distilled water was evaluated as the placebo group which investigated *L. nilotica* using anti-leech assay. Then extract and drugs were added and their effects were screened for 720 min and time to paralyze, kill and death of each leech was recorded. The results showed that olive methanolic extractions (600 and 300mg) could kill the leeches in an average time of 145±77.57 and171±33.28 min, respectively. An average death time for levamisole was found to be 15±7.49 min. The highest effectiveness was found for levamisole at dose 300 mg. Methanol extracts of the Harmal (300 and 600 µg/m) and springs water showed no anti-leech. In sum, olive plant could use for anti *Limnatis nilotica* expenditure.

KEY WORDS: *Peganum harmala* L., *Olea europaea* L., Levamisole, *Limnatis nilotica*, Anti-leech Assay

INTRODUCTION

Leeches that cause parasitic pollution in humans are terricolous or aquatic. Terricolous or land leeches include *Haemadipsa zylanica*, *Haemadipsa sylvestris*, and *Haemadipsa picta*, while aquatic leeches include *Limnatis nilotica*, *Myxobdella africana*, *Dinobdella ferox*, *Phytobdella catenifera*, and *Teromyzon tessulatom* (Haycox *et al*., 1995; Vera *et al*., 2005; Wallis, 1988).

Leeches are not true worms, but they are described as parasitic worms. Leeches are segmented worms that belong to the phylum Annelida and comprise the subclass Hirudinea which are external parasites of humans, domestic and wild animals (Chares AND Hendrix, 1981).

They also have two suckers, with each sucker located at the end of each animal, which can suck blood of her host as many as nineteen times of body weight. Leeches as pathogenic parasites cause complications such as pain, itching, inflammation, severe anemia, short-term bleeding, hypersensitivity, and anaphylactic reactions on their host (Ahmadizadeh, 2001; El-Awad and K Patil, 1990; Hadrani, 2000). In some reports in humans, leeches as a foreign body caused acute bleeding of the alimentary system in two children, 2.5 to 3 years old (Hemmati *et al*., 2002), hematemesis in a 3.5-year-old child (Kazemi and Bajoghli, 2002) or bleeding of the vagina (Yaghmaee, 2002), and one case of

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ophthalmorrhagia that was caused by leech attachment has been reported (Davari, 2008). The parasite in the respiratory tract has been reported in animals. Some reports have mentioned that hirudiniasis may cause severe anemia with hemoglobin lesser than 5g/dl (Hemmati *et al*., 2002). Nowadays, hirudiniasis categorized as emerging and reemerging diseases (Bahmani *et al*., 2010).

According to World Health Organization (WHO), plants are supplier of medicines for human resources. Factors such as lack of access to the majority chemical drugs, cost of drugs, reduce the therapeutic effects of chemical drugs because of its resistance to pathogens and cause side effects, leading up to the day on traditional medicine and treatment plant societies to the positive more (Ghasemi pirbalouti, 2009).

Types of medicinal plants used in the treatment of various diseases are the olive and *P. harmala* L. Harmal is a plant of the family Nitrariaceae, native from the eastern Mediterranean region east to India. It is a perennial plant, which can grow to about 35-100 cm tall. The roots of the plant can reach a depth of up to 6.1 m, if the soil it is growing in is very dry. The flowers are white and are about 2.5–3.8 cm in diameter .The round seed capsules measure about 1–1.5 cm in diameter, have three chambers and carry more than 50 seeds. In Turkey *Peganum harmala* is called yüzerlik or üzerlik and ispand in Iran, Afghanistan, Azerbaijan, Uzbekistan and Tajikistan, harmal in Iraq and alharma in Spain is called (Zarzuelo *et al*., 1991). *P. harmala* is used as an analgesic and anti-inflammatory agent and it was used to treat depression. It has antibacterial activity against drug-resistant bacteria and used treatment of syphilis in India, fever in North Africa, hysteria, neuralgia, Parkinson's disease, uterine prolapsed, rheumatism, asthma and eye irritation. *P. harmala* is an abortifacient and effective against protozoa including malaria (Markin *et al*., 2003).

The active alkaloids of Harmal seeds are the MAOI-A (monoamine oxidase inhibitor A) compounds. Studies in Pakistan and Egypt shows that the *P. harmala* extract revealed antifungal and antimicrobial effects and its effects are related to harmine alkaloids (El-Awad and Patil, 1990; Ahmad *et al*., 1992; Ross, 1980).

Olive tree or shrub of the family Oleaceae and evergreen stands, native to the coastal areas of the eastern Mediterranean Basin as well as northern Iran at the south end of the Caspian Sea, up to 20-10 cm high, often branching ramose and, with bayonets rectangular with oval leaves, white flowers, immature fruit is green and the shaft it is purple when ripe (Mozaffarian, 2008). The relaxation of blood vessels [14] and antioxidant properties of olive also had been recorded (Briante, 2002; Visioli and Galli, 1997).

Levamisole was originally used as an anthelminthic to treat worm infestations in both humans and animals. Most current commercial preparations are intended for veterinary use as a de wormer in cattle, pigs, and sheep. Levamisole stimulates the parasympathetic and sympathetic Ganglia in susceptible worms and interferes with nematode carbohydrate metabolism by blocking fumarate reduction and succinate oxidation. The net effect is a paralyzing effect on the worm which is then expelled alive. Levamisole's effects are considered to be nicotine-like in action.

Because the usefulness of medicinal plants must be proven through scientific assessments and research, in present study tried to evaluating the anti-leech effects of methanolic extracts of *Peganum harmala L.* and *Olea europaea L*. on *Limnatis nilotica* immature form.

MATERIAL AND METHODS

In this study, a number of immature form *L. nilotica* leeches (55 in total) were selected from the springs of the southern region of Ilam province (West of Iran). The dark green color surface with rows of green spots on the dorsal surface, and yellowish-orange and dark green bands on either side were the main signs for detection of *L. nilotica* species. The methanolic extractions of *Peganum harmala L.* and *Olea europaea L*. were prepared by adding a 1:3 ratio of olive and methanol, respectively, and this was subjected to Soxhlet extraction for 72 h according to the prescribed method of Eidi *et al*. (2006). After extraction, the solvent was filtered. The information studied for the plants has been determined in Table 1. Levamisole (Sinadaru, Iran) tablets (an anthelmintic and immunomodulator) were investigated as a control and compared with distilled water. These tablets were powdered and diluted in 10 ml distilled water. For the anti-leech assay, the leeches were located individually in a glass container with 600 ml spring water. The extract and drugs were then added, their effects were screened for 720 min, and time to paralyze, kill, and death of each leech was recorded.

The evaluation of death of a leech was based on immobility after stimulation with a needle. The low average paralyzing and killing time of these compounds reflects anti-leech properties (Bahmani *et al*., 2012). The severity of effect of these compounds/drugs based on time was categorized into five groups (Bahmani *et al*., 2012).

Classification of the intensity effect treatment

4+ is paralysis and death of each leech within 1–60 min after addition of the drug
3+ is paralysis and death of each leech within 61–120 min after addition of drug
2+ is paralysis and death of each leech within 121–180 min after addition of drug
1+ is paralysis and death of each leech within 181–240 min after addition of drug
Is negative paralysis and death of each leech within 241–720 min after addition of drug

The efficacy of the drugs which were able to kill leeches within 1–60 min after addition reflects the anti-leech properties of these compounds, and therefore, they may be used in the treatment of infestation with *L. nilotica* in the future (Bahmani *et al*., 2012). The differences between the control and treated groups were analyzed using one-way ANOVA using Sigma State 2 program.

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RESULTS

The anti-leech activities for treatments on *L. nilotica* are shown in Table 1. Paralysis and death properties of olive extraction according to criteria revealed that the methanol extract at different doses have different potential which increasing with dose. Methanol extracts of this plant had no effect *L. nilotica*. The highest affectivity was found for levamisole. The exact timing of drug treatments for paralysis and death are shown in Table 1. Statistical analysis shows the different drug treatments for paralysis and death factors, there is significant difference between the leeches.

Table 1. The anti-leech activity of used compounds compared to levamisole as positive control.

Extract/Drug	Mean ± SD	Dose	Severity
Levamisole	15±7.49	300mg/ml	4^{+}
Spring water	720±0.00	100 ml	-
Methanol extract of the Harmal	720±0.00	300 mg /ml	-
Methanol extract of the Harmal	720±0.00	600 mg /ml	-
Methanol extract of the Olive	171±33.28	300 mg /ml	2^{+}
Methanol extract of the Olive	145±77.57	600 mg /ml	2^{+}

DISCUSSION

The results of this study showed that olive methanolic extractions (600 and 300mg / ml) could kill the leaches in an average time of 145±77.57 and171±33.28 min while average death time for levamisole was found to be 15±7.49 min. The highest effect was found for levamisole (300 mg/ml). Methanol extracts of the Harmal (300 and 600 mg) and spring water showed no anti-leech effect. Therefore, we recommend that the other plant extracts (ethanol, hexane, hydroalcoholic) be examined on leech species.

Olive leaf contains choline compounds, unsaturated lactones, elenolide, oleuropeic acid , sugar, tannin, wax, manit and gallic acid have been identified (Sanei, 1970). The oleuropine components contain biological properties, barrier properties and the ability to activate free radical oxidation (Visoli *et al*., 1997 and 1998). Oleuropein, have pharmacological properties, which have an antibiotic and antiviral effects. Other, compounds of olive contains oleoropein, caffeic acid, elenolic acid glucoside and luteolin 7-glucoside (Amiot, 1998).

In present study, increasing doses of methanol extracts of the olive from 300 mg to 600 mg caused reduction in the average death time by 26 minutes, which revealed that ability of anti-leech effects enhance with increasing doses or concentrations of metabolic substances. This result- suggested that another components of olive be identified and examine on mature form of *L.nilotica*.

Bahmani *et al*. (2011) reported that methanolic extract of *Allium sativum* L. exhibited anti leech activities on *l. nilotica* immature form. They added that the average time of leech death (Immature form) for *Allium sativum* L was 68.44±28.39 min and for niclosomide was 6.22±2.94 min (Bahmani *et al*., 2011). Gholami-Ahangaral *et al.* (2012) studied effects of the methanolic extract of *Vitis vinifera L*., niclosamide and ivermectin on *l. nilotica* and found that mean death time of leeches treated with niclosamide and ivermectin for mature and immature forms were 15.4 and 11.2 and 10.1 and 11.2 minutes, respectively.The doses of 300 and 600 mg of methanol extract of *V. vinifera* L. against *L. nilotica* mature worm were ineffective but they exhibited death time with 260±63 and 200±50 minutes, respectively against the immature form of *L. nilotica*.

In a study by Bahmani *et al*. (2012) It was found that J. regida and N. oleander have no anti leech effect while Piperazine, Praziquantel, Chlorquine, Levamisole, Niclosamid, Copper sulfate and Chlor killed leeches at 720±0, 40.22±19.05, 720±0, 19.22=8.82, 17.11-4.13, 16.25±6.49 and 6.75±2.98 minutes after exposure, repectively. Bahmani *et al.* (2012) in another study evaluated effects of the methanolic extract of *Peganum harmala L*. and some of the anti parasite drugs on *L. nilotica* (Mature forme).The mean death time of leeches in groups treated with niclosamide, sulfadimidine, furazolidone and pyrvinium were 14.77±3.66, 58.33±22.17, and 137.11±37.84 and 320.44±300 min, respectively. Different doses of *P. harmala* L. methanolic extract (300, 600, 900, 1200, 1500 and 1800 mg) were ineffective on mature leeches in 720 min of experiment (Bahmani *et al*., 2012). In this study, Harmal also not any effect in immature forme of leeches. In another study by Gholami-Ahangaran *et al*., 2012 the results that study was indicated which *Olea europaea* L. plant and levamisole have more effect on the mature worm of *L. nilotica*. The mean death time of leech for levamisole and Olive determined 10±0.98 and 210±24.1 minutes, respectively. In our study, *Olea europaea* L. methanol extract had also good effect of anti leech of immature worm of *L. nilotica*.

It seems that Iranian Medicinal Plants, Sometimes may be having anti leech effects which due to the active ingredients in these herbs.

CONCLUSION

The survey results showed that methanol extracts of the harmal had no anti leech effects, and Olive has a weak effect on L.*nilotica* and using of it as a powerful and effective medicinal plant natural product is not recommended but can use as an effective supplement for medicinal products.

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