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## Comparative and evaluation of anti-leech (*Limnatis Nilotica*) effect of Olive (*Olea Europaea* L.) with Levamisol and Tiabendazole

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## ABSTRACT

**Objective:** Until now, there is no registered drug for treatment of complications with leech in the world. According to the available scientific evidence, Olive is an effective anti-parasitic plant. Hence, in the present experiment we studied the inhibitory and killing effect of Olive methanolic extract on *Limnatis nilotica*. **Methods:** In this study, 100 leeches (*Limnatis nilotica*) were collected from some wells in western area of Iran (south region in Ilam province) and evaluated the anti- leech effects of Olive methanolic extract (*Olea europaea* L.) in comparison with levamisole and tinidazole. **Results:** The results indicated no effect of tinidazole and distilled water on killing or mortality rate of the leeches but *Olea europaea* L. plant and levamisole have more effect on the *L. nilotica*. The mean death time of leech for levamisole and Olive determined  $10 \pm 0.98$  and  $210 \pm 24.1$  minutes, respectively. **Conclusions:** The results showed that treatments of Olive methanolic extract and levamisole have the most effects on leeches and could be used as natural anti-*L. nilotica*. However it is necessary to achieve further studies for confirm of this subject.

### 1. Introduction

Leeches that cause parasitic infections in humans are terricolous or aquatic. Terricolous or Land leeches include *Haemadipsa zylatica*, *Haemadipsa sylvestris*, and *Hamadipsa picta*, while Aquatic leeches include *Limnatis nilotica*, *Myxobdella Africana*, *Dinobdella ferox*, *Phytobdella catenifera*, and *Teromyzon tessulatom*<sup>[1–3]</sup>. Leeches as a pathogenic parasites cause complications such as pain, itching, inflammation, severe anemia, short-term bleeding, hypersensitivity and anaphylactic reactions<sup>[4]</sup>.

Olive is a member of the Oleaceae which is a long-lived evergreen tree. The Olive tree is an evergreen tree native to the Mediterranean, Asia and Africa. It is short and squat, and rarely exceeds 12–15 meters in height. The important parts of this plant include fruit and leaf were also used in pharmacotherapy against parasites<sup>[5]</sup>. Based on ethnobotanical information the methanolic extract from fruit and leaf of Olive (with scientific name of *Olea europaea* L. and Persian name of Zeytoon) suggested in traditional

medicine as antiparasite<sup>[6]</sup>. Also, Tinidazole is an anti-parasitic drug used against protozoan infections. It is widely known throughout Europe and the developing countries as treatment for a variety of amoebic and parasitic infections. It is chemically similar to Metronidazole<sup>[7]</sup>. Levamisole is an anti-helminthic and immunomodulator belonging to a class of synthetic Imidazothiazole derivatives<sup>[7]</sup>. Additionally, Levamisole stimulates the parasympathetic and sympathetic ganglia in susceptible worms and interferes with nematode carbohydrate metabolism by blocking fumarate reduction and succinate oxidation<sup>[7]</sup>. It has a paralyzing effect on the worm which is then expelled alive. Levamisole effects are considered to be nicotine-like in action<sup>[8]</sup>. Thus, in the present study, the anti leech effect of Olive methanolic extract (*Olea europaea* L.), Levamisole and Tinidazole was evaluated.

### 2. Materials and Methods

#### 2.1. Preparation of the herbal plant extract

Olive (*Olea europaea* L.) was identified by the Department of Botany of the Science Faculty, Tehran University, Iran.

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The methanolic extract of Olive leaf and fruit was prepared in Department of Botany, Science Faculty, Tehran University (Iran) on July 2011. Firstly, the olive Leaf and Fruit were powdered by the blender and then 100 gram of dried plant powder was soaked with 300 ml methanol (37 degree) in percolator for 48 hours in 50 centigrade degree in the oven[9]. Finally, dried extract was prepared from olive plant.

## 2.2. Preparation of the leech (*Limnatis nilotica*)

In this experimental study, 100 *Limnatis nilotica* leeches with 60–75 mm length were selected from spring waters from south region in Ilam province. The strong jaws and muscular suckers at the anterior and posterior ends, dark green color surface with rows of green spots on dorsal surface and yellowish–orange and dark green bands on either side with 30–100 mm length were the main signs for detection *Limnatis nilotica* species.

## 2.3. Preparation of the chemical drugs

In the present study, Tinidazole (Pars Daru, Iran) and Levamisole (Sina Daru, Iran) were investigated as a positive control and compared with distilled water and plant drugs. These drugs were powdered and diluted in 10 ml distilled water.

## 2.4. Experimentation

### 2.4.1. Anti-leech assay

Firstly, leeches were put individually in a glass container with 600 ml spring water. Then extract and drugs were added and their effects were screened for 720 min and the time of paralysis and death of each leech was recorded. The examination was repeated for nine times. The evaluation of death of leech was based on immobility after stimulation with needle. The low average paralysis and death time of these compounds reflect anti leech properties [8, 10,11].

The severity of effect of these compounds/drugs based on time categorized five groups:

- 1) 4+ <sup>H</sup> paralyze and death of each leech within 1–60min after addition of drug
- 2) 3+ <sup>H</sup> paralyze and death of each leech within 61–120min after addition of drug
- 3) 2+ <sup>H</sup> paralyze and death of each leech within 121–180min after addition of drug
- 4) 1+ <sup>H</sup> paralyze and death of each leech within 181–240min after addition of drug
- 5) Negative <sup>H</sup> paralyze and death of each leech within 241–720min after addition of drug[8, 10,11].

The efficacy of drugs, were clarify based on killing rate of leeches within 1–60 min after addition of drug. The killing rate reflects anti-leech properties of one compound and therefore, this compound may be used in the treatment of infestation with *L. nilotica* in the future [8, 10,11].

### 2.4.2. Analysis

The differences between the control and treated groups were analyzed using one-way ANOVA statistical method by Sigma State 2.0 software.

## 3. Results

The extract of *Olea europaea* L. showed anti leech activities (1+ severity). Among the treatments tested levamisole with 4+ severity and extracte of *Olea europaea* L. with 1+ severity showed the best anti *Limnatis nilotica* activity. The result showed that Tinidazole and distilled water with negative severity, have no effect on *L. nilotica*. The detail of results mentioned in table 1.

**Table I**

The characterizes of Treatment, Dose (mg), Mean±SD and Severity

Severity	Mean ± SD	Dose (mg)	Treatment
4+	10±0.98a	100	Levamisole
–	720±0c	500	Tinidazole
–	720±0c	100	Distilled water
1+	210±24.1b	600	<i>Olea europaea</i> L.

\*The mean death time presented as Mean±SD.

a,bDifferent words in each column represent existance of significant differences between groups.

## 4. Discussion

The strongest anti leech effect was shown by Levamisole and the extract of *Olea europaea* L.(Olive). Based on the results of this study, the methanol extract of Olive was significantly more active against *L. nilotica* after Levamisole. Tinidazole and distilled water were determined ineffective.

The medical effects of Olive leaf extract were included diuretic, anti hyperglycemic, anti cholesterol, anti acid uric, anti hypertension and anti atherosclerosis. Additionally, Olive leaf extract was prevented insulin resistant in laboratory animals and cause decreasing in blood Glucose level. In traditional medicine and health mentioned that Olive can be used in treatment of hypertension, atherosclerosis, urinary system infections, headache and fever [12–16].

Zargari reported that Olive extract is effective on malaria [5]. Bahmani et al. (2010) were studied the anti-*Limnatis nilotica* effects as well as tobacco methanol extract and some other anti-parasite drugs such as Mebendazole, Metronidazole, Triclabendazole, Levamisole, Niclosamide, and Succinylcholine [10]. At previous study tobacco methanolic extract (600 mg) was enable to kill the leeches in the average time of 17±6.11 min. Also, in another study, Bahmani et al. (2010) were evaluated the anti-*Limnatis nilotica* effects of eight anti-parasite drugs, comprised of Closantel, Ivermectin, Niclosamide, and Levamisole. These drugs were have killing effect with 4+ severity, while Triclabendasole

and Albendasole have 3+ and 2+, respectively<sup>[11]</sup>. But present study was the first report of anti *Limnatis nilotica* effect of Olive plant.

According to study of Farkhondeh et al. (2010), the average time of killing of leech for Levamisole was determined 7 min and for garlet tablet were negative severity<sup>[18]</sup>.

In another study by Eftekhari et al. (2011), the average time of paralysis and the death of *Limnatis nilotica* for Metronidazole, the methanolic extract of *Allium sativum* L. and Levamisole was 718.77±66.3 min, 5.11±1.76 min and 144.55±57.217 min, respectively. In this study, it was determined that Levamisole, with an intensity of 4+ and the methanolic extracts of *A. sativum* L. with an severity of 2+, have a good anti-leech effect and can be shown to be effective in cases of leech biting, while Metronidazole and distilled water were negative<sup>[8]</sup>. In the present study, anti- protozoa drug such as Tinidazole (500 mg ) did not have any anti-leech effect but in previous studies by Bahmani et al in 2010 and Eftekhari et al. in 2011, Metronidazole had moderate anti- leech effect with dose 125 and 250 mg <sup>[8,11]</sup>.

These results corroborate the importance of ethno-pharmacological surveys in the selection of plants for bioactivity screening. The results obtained in the present survey were a worth while, expressive contribution to the characterization of the anti-Leech activity of this plant extract of traditional medicinal plants from the Iranian flora. Subsequently, bio-guided fractionation will be conducted on plants showing potential anti-leech activity to identify the active compounds. Evaluations of the extract against other important human and animals pathogens are also being conducted.

## Conclusion

The results showed that treatments of Olive methanolic extract and levamisole have the most effects on leeches and could be used as natural anti-*L. nilotica*. However it is necessary to achieve further studies for confirm of this subject.

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