

Evaluating the Anti-Leech Activity of Methanolic Extract of *Matricaria chamomilla* L. Comparing with Ivermectin, Mebendasole, Praziquantel, Rafoxanide, Febantel and Albendasole

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Abstract: Common features of leeches are nature parasitic and most important complication with infestation in host is anemia. In different Nations Pharmacopeia not registered drug for treatment of complications with leech in the world. This study prepared methanolic extract of *Matricaria chamomilla* L and several selected chemical drugs such as Febantel, Mebendazole, Rafoxanide, Praziquantel and Ivermectin and studied on leech *Limnatis nilotica*. In July 2010, in the southern area of Ilam province, 80 *Limnatis nilotica* leeches were collected and evaluated by anti leech assay. *Matricaria chamomilla* L killed leeches after 232.22 ± 45.19 minutes. The mebendazole (500 mg), praziquantel (50 mg) and rafoxanid (150 mg) could not kill the leech but febantel with dose 100 mg caused death after 494.74 ± 337.9 minutes. Ivermectine had best effect on leech. Lack of death leech was monitored by mebendazole, praziquantel and rafoxanid. The results of present study showed that *Matricaria chamomilla* might be used per se or as complementary treat in leeches' infestation.

Key words: Leech % *Limnatis nilotica* % Medicinal Plants % Anti Parasitic Drugs % *Matricaria chamomilla* L.

INTRODUCTION

Leeches, a hermaphroditic, blood-sucking parasite are rarely reported in humans and animals as a cause of many problems. Land leeches include *Haemadipsa zylani*, *Haemadipsa sylvestris*, *Hamadipsa picta*, while Aquatic leeches include *Limnatis nilotica*, *Myxobdella africana*, *Dinobdella ferox*, *Phytobdella catenifera* and *Teromyzon tessulatom* [1-3].

The leeches mainly inhabit in ponds, lakes and streams. The *Limnatis nilotica* species are blood-sucking parasite that lives in stagnant water in ponds and lakes. The strong jaws and muscular suckers at the anterior and posterior ends of *Limnatis nilotica* are the main sign for detection. This species inhabit commonly in Southern Europe, North Africa and the Middle East including Iran [4]. These leeches live in hosts and can cause anaemia

and may act as vectors of animal pathogens. The main symptoms included pain, haemoptysis, snoring, dyspnea, cough, dysphagia, anaphylaxis and bleeding from vagina [5-6]. Serious complications are expected like dyspnoea, hemoptysis [7] or hematemesia and bleeding from vaginal [8]. The leeches can transmit some bacteria, viruses and parasites. Some lethal diseases such as AIDS, Hepatitis B and Toxoplasmosis can be transmitted by leeches [9-11]. In some cultures for removing leech have been used from Hypertonic fluids, acid acetic and Lidocaine [12]. As no effective drug, without any side effect and with appropriate cost benefit, has been offered for leeches, we decided to study the anti-leech (*Limnatis nilotica*) effects of some herbal and chemical compounds. Chamomile is one of the most widely used and well-documented medicinal plants in the world. The use of chamomile as a medicinal plant dates back to ancient

Greece and Rome. *Matricaria chamomilla* L., locally known as “Babouneh” is widely distributed throughout all regions of Iran and is natively found on the road-side and unused fields. In medical used *M. chamomilla* L. for Bronchitis, Eczema, Psoroptiasis, Anti cough, Anti spasmodic and alleviated pain. Additionally, *M. chamomilla* has anti microbial and anti inflammation effects [13-17] and in some regions of Iran used as anti-leech. Thus, in the present study, we investigated the possible anti -leech activities of methanolic extract obtained from the aerial part of *M. chamomilla* L.

MATERIALS AND METHODS

Leech Preparation: In this study, 80 *Limnatis nilotica* leeches with 50-70 mm length, were selected from spring waters from south region in Ilam province (west of Iran). 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 30-100mm length were the main sings for detection *L. nilotica* species.

Preparation of the Plant and Extract: The information of the medicinal plant used in the Table (1) bellow. The plant was identified by the Department of Botany of the Science Faculty, Tehran University, Iran.

Air-dried *M. chamomilla* L. was pulverized with a blender. One hundred grams of this plant material was extracted on a Soxhlet apparatus using 11 of Methanolic solvent containing 37% methanol and 63% distilled water. Finally, the solvent was recovered and the extract was lyophilized, weighed (yield: 17.7%). The extract (MCE) was further diluted with distilled water to obtain different doses [19].

Preparation of the Chemical Drugs: The drugs comprising Ivermectin (Injectable form 1%; Erfa-daru co., Iran), Mebendasole (Bolos 500 mg; Damloran co., Iran), Praziquantel (Pill 50 mg; Damloran co., Iran), Rafoxanide (Bolos 150 mg; Damloran co., Iran), Febantel (Pill 100 mg; Damloran co., Iran) and Albendasole (Bolos 600 mg; Darohaye-dami co., Iran) were investigated and compared with distilled water as negative control. These drugs (Ivermectin, Mebendasole, Rafoxinide, Praziquantel, Febantel and Albendasole) were powdered and diluted in 10 ml distilled water then were added to glass containet.

Anti-Leech Assay: First, leeches were put individually in a glass container with 600ml spring water. Then extract and drugs were added and their effects were screened for

Table 1: Information of the used plant

Scientific Name	Local Name	The used Part	Type of extract	Traditional Treatment [18]
<i>Matricaria chamomilla</i> L.	chamomilla	Flower	Methanolic extract	Anti-parasite

720 min and time to paralyze kill 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 shorter average paralysis and killing time of these compounds reflected their effectiveness as drugs with anti-leech properties. Leeches to be killed in sooner time, the drug is stronger and effective [25]. The severity of effect of these compounds/drugs based on time categorized five groups:

- C 4*6 paralyze and death of each leech within 1-60min after addition drug.
- C 3*6 paralyze and death of each leech within 61-120min after addition drug
- C 2*6 paralyze and death of each leech within 121-180min after addition drug
- C 1*6 paralyze and death of each leech within 181-240min after addition drug
- C Negative6 paralyze and death of each leech within 241-720min after addition drug [25].

The efficacy of drugs which could kill leeches within 1-60min after addition and reflect anti leech properties of these compounds and therefore they may be used in the treatment of infestation with *L. nilotica* in the future [25]. The differences between the control and treated groups were analyzed using one-way ANOVA and Sigma State 2 program.

RESULTS

The mebendazole (500 mg), praziquantel (50 mg) and rafoxanid (150 mg) could not dead the leech but febantel with dose of 100 mg with mean time of 440.44 minutes was killed the leeches. Ivermectine with time 21.55 min (4*) had best effect on leech (Table 2).

The results of this study showed that methanolic extract of *M. chamomilla* L (600mg/ml) could kill the leaches in the average time of 232.22 min (1*). Average death times for other drugs (Ivermectin, Mebendasole, Praziquantel, Rafoxanide, Febantel and Albendasole) were found 21.55 (4*), 720 (-), 720 (-), 720 (-), 494.74(-) and 138(2*)min, respectively. The highest affectivity was found for Ivermectin.

Table 2: Mean death time following indication of different drugs

Treatment	Dose (mg/600ml)	Mean±SD	Severity
Ivermectin	10	21.55±2.71 ^d	4 ⁺
Mebendasole	500	720±0 ^a	-
Praziquantel	50	720±0 ^a	-
Rafoxanide	150	720±0 ^a	-
Febantel	100	494.74±337.9 ^b	-
Albendasole	600	138±69.34 ^c	2 ⁺
Distilled water	100	720±0 ^a	-
<i>Matricaria chamomilla L</i>	600	232.2±45.19 ^c	1 ⁺

C The different superscripts (a, b, c and d) in column represent existence of significant differences between groups (P<0.05)

DISCUSSION

In some cultures for alleviating pain of Hirudiniasis have been used salt, sodium bicarbonate, tobacco leaf, calcium hydroxide and sedatives [13-17]. For finding effective drugs, specially herbal drugs, we decided to study the anti-leech effects of methanolic extract of *M. chamomilla* L. Ivermectin, Mebendasole, Praziquantel, Rafoxanide, Febantel, Albendasole and methanolic extract of *M. chamomilla* L (MCE) had anti-leech effects but Mebendasole, Praziquantel, Rafoxanide and Febantel didn't show any anti-leech effect. Treatment with different doses of MCE significantly reduced postprandial hyperglycemia and oxidative stress, anti inflammation, anti spasmodic, anti diarrhea and augmented the antioxidant system. MCE used for controlling some disorders as fever, acne, seizure, apnea and vomiting [13-17].

Dried flowers of *M. chamomilla* are largely used to provide sedative as well as spasmolytic effects. Throughout history, chamomile has been (and still is) used in a variety of ways: healing baths, teas, poultices, air fresheners, hair rinse, cosmetics, insect repellents, wine flavoring. Chamomile is medically specific to be useful with all of the following: soothing, calming, sedative, relaxation, anti-inflammatory, tension, aching muscles, indigestion, acidity, hay fever, asthma, morning sickness, eczema, sore nipples and exhaustion [20]. Chamomile is known for its calming effect on smooth muscle tissue and is still a popular remedy for nervous stomach, menstrual cramps and other common problems related to stress [21].

Bahmani *et al.* in 2010 [22] studied the anti-leech effects of tobacco methanolic extract and also some other drugs such as mebendazole, succinylcholine, metronidazole, triclofenadazole, levamisole, niclosamide. The results of this study showed that tobacco methanolic extract (600mg/ml) could kill the leeches in the average time of 17 minutes. Average death times for other drugs (triclofenadazole, levamisole, niclosamide and metronidazole) were found 118.66, 7, 18.66 and 541.11 min, respectively [4]. Our study proved that results.

The antifungal activity of *M. chamomilla* L. flower essential oil was evaluated against *Aspergillus niger* [23].

Bahmani *et al.* [24] 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 niclosamide was 6.22±2.94 min. Gholami-Ahangaran [25] 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*.

Bahmani *et al.* [26] in another study evaluated effects of the methanolic extract of *Peganum harmala* L. and some of the anti parasite drugs on *L. nilotica* (Mature form). The mean death time of leeches in groups treated with niclosamide, sulfadiazine, furazolidone and pyriminyl 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.

Results of this study showed no effect of mebendazole, praziquantel and rafoxanid in leech death time but Methanolic Extract of *Matricaria chamomilla* L. could be had partial effect on leech death time. Therefore *Matricaria chamomilla* L. could be suggested as anti leech herbal drug. The results of present study showed that *Matricaria chamomilla* might be used per se or as complementary treat in leeches' infestation.

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