

THE ANTI-LEECH EFFECT OF ETHANOLIC EXTRACT OF *ACHILLEA MILLEFOLIUM* L. COMPARED TO LEVAMISOLE AND NICLOSAMIDE ON *LIMNATIS NILOTICA*

Mahmoud Bahmani¹, Fatemeh Abdi², Ahmad Adineh¹, Hassan Hassanzadazar³, Bahareh Eghbali⁵, Majid Gholami-Ahangaran⁴, Mahmoud Rafieian-Kopaei⁶

¹Razi Herbal Medicines Research Center, Lorestan University of Medical Sciences Khorramabad, Iran

²Islamic Azad University, Science and Research, North Tehran Branch, Tehran, Iran

³Deputy for Food and Drug, Urmia University of Medical Sciences, Urmia, Iran

⁴Poultry Diseases Department, Veterinary Medicine Faculty, Islamic Azad University, Shahrekord Branch, Shahrekord, Iran

⁵Faculty of Pharmacy, Isfahan University of Medical Sciences, Isfahan, Iran

⁶Medical Plants Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran

Abstract: This experimental trial was designed to evaluate the anti-*Limnatis nilotica* effect of *Achillea millefolium* L. ethanolic extract as well as levamisole and niclosamide. In an experimental study the extract of *Achillea millefolium* L. aerial parts was prepared and then the severity effect of the treatments was recorded and compared with placebo group on *L. nilotica* as anti-leech assay. The average time of paralysis and death of *L. nilotica* for Levamisole, niclosamide and *Achillea millefolium* L. plant were 12.66±5.19 min, 19.22±3.42 min and 90±17 min, respectively. Distilled water was determined as the inert for control group. In this study, it was determined that *Achillea millefolium* L. plant with an intensity of 3+ have a good anti-leech effect and can be shown to be effective in cases of leech bitings.

Keywords: Leech, *Limnatis nilotica*, *Achillea millefolium* L., Ethanolic extract

INTRODUCTION

Parasites and parasitic diseases as a challenge in health systems have widespread outbreaks around the world. Poor health, illiteracy, use of contaminated resources and lands, use of contaminated foods and drinks, poverty, malnutrition and overpopulation are the main factors for contamination (Bahmani *et al.* 2014; Collier *et al.* 1998). Parasitic infections cause diarrhea, anemia, digestive problems, weight loss, poor concentration, abdominal pain and loss of function (Okay *et al.* 2004; Steven *et al.* 2003). Parasites are classified into various categories such as cestodes, trematodes, nematodes and pseudo-parasites. Leeches as one of the parasites are classified into two groups: aquatic leeches and earthworms. *Limnatis nilotica* is the main species that contaminates both humans and animals. Dark green leeches usually have 1 cm length, two anterior and posterior suckers and orange lines on both sides of the body. The main complication of leech infestation include anemia, weight loss, respiratory distress, restlessness, haematemesis, haemoptysis, bleeding, vaginal, mouth and rectal bleeding and etc. (Bahmani *et al.* 2013; Bahmani *et al.* 2013). Nowadays, there is great attention to treatment with plants. Treatment with plants as a branch of traditional medicine in countries with old civilization like Iran has already played a major role in the treatment of diseases (Fong HH, 2002).

Now in many developed countries, the use of medicinal plants and traditional medicine to treat many diseases including experimental wound healing, hypertension, diabetes, reproductive tract function, parasites and many other effects is popular (Rafieian-Kopaei, 2012). In recent years, the use of herbal medicines and herbal origin is increasing about one

third to one half of the pharmaceutical products in the United States, are plant origin [14].

Yarrow is a plant with the scientific name *Achillea millefolium*, with rhizome and one meter height direct stem. Consumable parts of the plant are blooming branches with bitter taste and strong odor collected in summer during flowering (Valnet J 2002; Zargari A. 1988).

Main medicinal applications of yarrow are: bleeding stopping, sudatory, growth stimulating, healing of gastrointestinal discomfort such as diarrhea and bloating, antibiotics, reducing fever, treatment of insomnia, strengthening the liver, treatment of liver cirrhosis, hepatitis, arthritis, lungs bleeding. It is also used as a mouthwash, rapid treatment in cold, expectorant, healing of wounds and old scars, suitable treatment to reduce blood urea and mucus in bladder (Spriggs D, 1998).

The yarrow is known as an antiparasitic plant in Iranian traditional medicine (Ghasemi A, 2011). At the moment, there is no study regarding the effects of this plant on leech. The aim of this study was to evaluate the effect of this plant ethanol extract on *Limnatis nilotica*.

MATERIALS AND METHODS

Preparation of leeches

In this experimental trial, in august of 2014, a total of 57 *Limnatis nilotica* (Leech) were collected from spring water of Dehloran city, Ilam province, west area of Iran.

Extract preparation

In this experimental study, the plant *Achillea millefolium* was prepared from Dehloran city of Ilam

province in the west of Iran and the species was authenticated. Collected Yarrow flowers were dried and powdered with a mixer. Then 50 g of the dried powder was mixed with 500 ml of 96% ethanol. The solution was filtered through Buchner funnel after 72 hours and then the solution was concentrated and the resulting material was dried using nitrogen gas. The considered dose was prepared using saline and adding TWEEN- 40 (Biogene Company) to resulting suspension (Parandin *et al.*2011).

Experimentation of anti-leech assay

Leeches were put individually in a glass container with 600 ml spring water. Then, the extract and drugs were added and their effects were screened for 720 min and the time to paralyze or kill each *L. nilotica* 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 paralyzing or killing time of these compounds reflects anti leech properties.

The severity effect of these compounds/drugs based on the time was categorized in five groups as follows:

- 1) 4+→ paralyze and death of each leech within 1-60 min after addition of drug.
- 2) 3+→ paralyze and death of each leech within 61-120 min after addition of drug.
- 3) 2+→ paralyze and death of each leech within 121-180 min after addition of drug.
- 4) 1+→ paralyze and death of each leech within 181-240 min after addition drug.
- 5) Negative→ paralyze and death of each leech within 241-720 min after addition of drug.

The efficacy of drugs which could kill the *L. nilotica* within 1-60 min after addition reflects anti leech properties of these compounds and therefore they may be used in the treatment of infestation with *L. nilotica* in the future.

Statistical analysis

The differences between the control and treated groups were analyzed using one-way ANOVA and Sigma State 2 program.

RESULTS AND DISCUSSIONS

Anti-leeches tests were reported based on the mean time to death with nine times repetitions and as mean±standard deviation. In this study, levamisole had the most effective Anti-leech activity. Niclosamide killed leeches after 19.22±3.42 Min. It was found that yarrow had strong effective medicinal influence with 3⁺ on leeches. Detailed information of various treatments is shown in Table 1.

Table 1.

Treatment	Dose (mg)	Mean±SD (min)	influence
Yarrow ethanol extrac	600	90±17 ^a	3 ⁺
Levamisole	600	12.66±5.19 ^b	4 ⁺
Niclosamide	600	19.22±3.42 ^c	4 ⁺
Distilled water	100	720±0 ^d	-

Test between all groups

Due to non-normality of the data based on the Kruskal-Wallis, One Way Analysis of Variance on 1 Anjam and all groups using Dunnet with distilled water as a control were compared and significant differences between the control group and all groups were observed ($p < 0.001$).

Test between the main groups (Yarrow, Levamisole and Niclosamide)

Due to non-normality of the data based on the Kruskal-Wallis One Way Analysis of Variance on Ranks was performed, and all groups were compared by Dunnet test and significant differences were observed between groups in all groups ($p < 0.001$).

Test between the levamisole and niclosamide

Method One way ANOVA was used to compare the groups and significant differences were seen by Tukey test, respectively ($P = 0.006$).

In this study, anti-leeches effect of ethanol extract of yarrow was studied in comparison with two strong antiparasitic drugs. The results showed that the paralysis and death effect on leeches were relatively strong in comparison to two anti-parasite drugs.

Several studies have been done on the effects of medicinal plants on leeches such as *Limnatis nilotica*. Results of a study showed that the methanol extract of garlic was very effective on leeches (Eftekhari *et al.*2012), while the methanol extract of harmala (300 and 600 mg), even at high doses (900, 1200, and 1500 mg) had no protective effect on *Limnatis nilotica* (Bahmani *et al.* 2012; Bahmani *et al.* 2012). Methanol extract of onion (600 mg) was ineffective on *Limnatis nilotica* leech species (Bahmani *et al.*2013). These results were not consistent with our results.

The findings of several other studies were consistent with our results. Studies showed that methanol extract of tobacco (600 mg) and its nicotine as active ingredient (5, 10, and 20 mg) killed leeches with the 4+ intensity and had strong effect on mortality of *Limnatis nilotica* (Bahmani *et al.* 2012; Bahmani *et al.* 2014). In another study, effects of methanol extracts of chamomile on leeches were reviewed and it was determined that a dose of 600 mg of it was not so strong for Leech death (Bahmani *et al.*2012). The methanol extract of Grape at doses of 300 and 600 mg at a mean time 260 ± 63 min (extremely negative impact) and 200 ± 50 min (intensity caused 1⁺) caused the death of Leeches (Gholami-Ahangaran *et al.* 2012). Olive methanol extract at a dose of 600 mg, with a mean time of 210 ± 24.1 minutes, caused paralysis and death of *Limnatis nilotica*, while levamisole treatment groups as control group caused leech death with 4⁺ influency. Olive has good effect on leech death compared with levamisole (Gholami-Ahangaran *et al.* 2012).

In this study, yarrow extract at a dose of 600 mg, with a mean time of 87 minutes, killed the leeches. Yarrow had strong effect on the *limnatis nilotica* leech with 3⁺ influence indicating a strong effect of this extract on leech.

The most effective ingredients in this plant (yarrow) include Cyanogenic glycosides (oxylein), choline, valeric acid, formic acid, tannins, camazolin volatile oil, alkaloids (Betonicine and stachydrine), flavonoids, resin, gum, amino acids, polyphenolic compounds, sesquiterpens, lactones, betaine, achylin, phosphate, nitrate, potassium salts and organic acids (Valnet J, 2002; Semsam Shariat H, 2007).

Apigenin, luteolin and Quercetin are as the main constituents of yarrow and are found in 7-O- glucoside and 7-Malunyl glycoside forms. Flavonoids are polyphenolic compounds that are found in all foods of plant origin with different effects on cellular system of mammalian (Melzig, M.F., 1996). Therefore, flavonoids may have a therapeutic effect on diseases caused by oxidative stress such as coronary atherosclerosis, ischemic injuries, diabetes mellitus, cancer and the aging processes (Haraguchi, H. *et al* 1996).

Antiparasitic (against leeches) effects of yarrow were identified in our experimental study. This study confirms the knowledge of traditional medicine in antiparasitic effects of yarrow (Haji Akhondi A. 2003; Blumenthal M. 2000). It appears that plant flavonoids and othe phenolic compounds are the main active ingredients. against leeches. In other words Apigenin, Luteolin and Quercetin may be affecting on leeches.

The results of an experimental study in a rat model showed that extracts and essential oils of the yarrow caused inactivation of *Enterobius vermicularis*, and cut off the worm expulsion (Izadi J *et al* 1382).

The results of other experimental studis in BALB/c mouse showed yarrow extract had a good influence on healing of cutaneous Leishmaniasis caused by major Leishmania (Izadi J *et al* 1382). In another study the effect of anti-parasitic effects of yarrow has been demonstrated on rural cutaneous leishmaniasis (Shirani-Bidabadi *et al* 2009).

It appears that anti *Limnatis nilotica* effects of yarrow is likely as a result of a cumulative effect of several active ingredients and Achylin, Thujone, Eugenole, Geraneol and Terpinol are applied more than other antiparasitic ingredients on leeches (Teyler *et al* 1988; Goldbray A, 2000).

Medicinal plants are a source of natural agents to treat diabetes (Bahmani *et al* 2014), and migraine headaches (Delfan *et al* 2014), antiparasites (Bahmani *et al* 2014, Saki *et al* 2014), psychiatric disorders (Saki *et al* 2014; (Bahmani *et al* 2014), respiratory diseases (Asadbeigi *et al*, 2014), toothache (Delfan *et al* 2014), treatment of skin disorders and wounds (Delfan *et al* 2014)¹ etc.

In general it can be stated that Yarrow is able to kill leeches and can be used as a natural anti-leech medicinal product.

ACKNOWLEDGEMENTS

The authors would like to appreciate financial support of Deputy for Food and Drug, Urmia University of Medical Sciences, Iran. Grant number of the research work is 34851.

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