

Full Length Research Paper

The anti-leech effect of *Peganum harmala* L. extract and some anti-parasite drugs on *Limnatis nilotica*

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Accepted 24 February, 2012

This experimental trial was designed to evaluate the anti-leech effect of methanolic extract of *Peganum harmala* L. plant as well as niclosamide, albendazole, pyrvinium, furazolidone and sulfadimidine. In an experimental study the methanolic extract of *Peganum harmala* L. aerial parts was prepared by maceration method and the amount of total phenolic compounds was determined colorimetrically using the Folin-Ciocalteu reagent. Then the severity effect of the compounds was recorded for 720 min and categorized in 5 groups (1 to 5), based on the time to paralyze or kill the leech. 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 extracts (300, 600, 900, 1200, 1500 and 1800 mg) were ineffective on leeches in 720 min of experiment. Despite of suggestion in Iranian traditional medicine related to the effect of *P. harmala* L. on leech infestation, the results of this experiment showed that *P. harmala* L. was ineffective. The niclosamide, sulfadimidine, furazolidone and pyrvinium might be used per se or as complementary treatment in leeches' infestation.

Key words: Leech, *Limnatis nilotica*, anti-leech assay, *Peganum harmala* L.

INTRODUCTION

Leeches (*Limnatis nilotica*) are 'worms' ranging in size from 0.5 centimeter to over 25 centimeters long. There are 650 known species of leeches. They often take in a lot of blood when they feed. Four or five large leeches can drain the life from a rabbit in half an hour (Eldor et al., 1996).

L. nilotica infestation primarily occurs in tropical areas, such as in Mediterranean countries, Africa and Asia (Uygur et al., 2003). Leeches have a mouth, three jaws with suckers which are specially adapted for attaching to and sucking blood from the skin and mucosal surface of mammalian species (Eldor et al., 1996). *L. nilotica* bites

on the skin are common, when people walk carelessly through bushes and water. Leeches have also been reported frequently in body cavities (Saha and Nagi, 2011). They use a proboscis to puncture the skin, or use their three mouths and millions of little teeth to do this, however; their bites are painless, due to its own anesthetic. After entering the mouth or nose of the host, they can cause bleeding and bloody nose, cough, difficulty breathing, pain and vomiting (Hendrix, 1998).

Leeches are usually taken into the human body while bathing or drinking unfiltered water or while swimming in contaminated water. They localize on the mucosa of the oropharynx, nasopharynx, tonsils, esophagus, nose, or in the rectal mucosa (Bilgen et al., 2002; Kaygusuz et al., 2001; Mohammad et al., 2002; Güloğlu et al., 2004).

As an endoparasite, *L. nilotica* can cause serious, even lethal, complications. In most of cases, it can cause

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severe morbidity mainly due to two factors, that is, mechanical obstruction of a vital organ, (Bergua et al., 1993) and/or severe bleeding.

Leeches can be removed mechanically or by medication. Since, it is attached too tightly to the mucosa and resists, forceful removal may cause severe and prolong bleeding. In these situations, hypertonic sodium chloride solution or cocaine/lidocaine can be applied, which releases the hold of the leech or paralyzes it, respectively. Afterwards, it can be removed easily (Pandey et al., 2000; Litch and Bishop, 2000). In case of endoparasitism, to avoid surgery we need a drug to kill the leech.

Peganum harmala L is a plant which is said to have anti-leech activity. The habitat of this plant is dry lands and plain regions in sandy soil. The primary origin of *P. harmala* L is central Asia but nowadays it grows in north of Africa, Australia and southwest of America. The main ingredients of *P. harmala* L are alkaloids localized in root and seed (Loub et al., 1985). The main indication of *P. harmala* L is as sedative and narcotic in traditional medicine. Also some evidence exists that this plant can be effective against worms. The *P. harmala* L indications in different parts of the world are varied. In India, it is used in syphilis treatment, in north of Africa is used for the treatment of fever. Also, this plant is used for the treatment of malaria, rheumatism, parkinson, asthma, hysteria, colic, uterus prolapse and eye infection (Glasby JS, 1987; El-Rifaie, 1980). The main alkaloids of *P. harmala* L seed are beta-carboline alkaloids such as harmalin and harmin and kinazoline alkaloids such as vesicinin and vesicinon (Glasby JS, 1978). The antibacterial and antifungal properties of *P. harmala* L are from harmin (Al-Sharma et al., 1981; Ross et al., 1980; Ahmad et al., 1992; Ghasemi-pirbalouti, 2009).

This experimental trail was designed to evaluate the effect of methanolic extract of *P. harmala* L as well as some synthetic anti-parasitic drugs including niclosamide, albendazole, pyriminyl, furazolidone and sulfadimidine on *L. nilotica*. This experiment was planned based on some reported effects of *P. harmala* L and some evidences of *P. harmala* L indication in leech infestation in Iranian traditional medicine.

MATERIALS AND METHODS

Ethno-botanical detail of the *Peganum harmala* L. and extraction

In July 2011, *P. harmala* L. with ethno-botanical detail mentioned in Table 1 was collected from Ilam region in Iran and authenticated in the department of botany in the Science Faculty of Tehran University. A voucher specimen of the plant was deposited in its herbarium department. The methanolic extract of *P. harmala* L. aerial parts was prepared by maceration method. First, the aerial part of this plant was powdered by the mixer (Molinex®, Italy) and 100 g of dried plant powder was soaked in 300 ml methanol 37% and left at room temperature for 48 h, then was filtered and the solvent was evaporated using a rotary evaporator. Finally, it was

dried in 50°C in oven.

Measurement of total phenolic compounds

The amount of total phenolic compounds in the *P. harmala* extract was determined colorimetrically using the Folin–Ciocalteu reagent, by the method of Francis (1982).

Five (5) mL of *P. harmala* extract or gallic acid (standard phenolic compound) was mixed with Folin–Ciocalteu reagent (1:10 diluted with distilled water) and aqueous Na₂CO₃ (4 mL, 1 M). The mixtures were allowed to stand for 15 min, and the total phenols were determined by colorimetry at 765 nm. A standard curve was prepared using 0, 50, 100, 150, 200, and 250 mg/L solutions of gallic acid in methanol: water (50:50, vol/vol). Total phenol values were expressed in terms of gallic acid equivalent (in mg/g). The experiment was repeated in triplicate.

Preparation of leeches

In summer of 2011, a total of 60 leeches were collected from some water wells around Dehloran city, Ilam province, west area of Iran. The leeches were *L. nilotica* and had some morphological properties that comprised of dark green color, orange longitudinal lines in body, small anterior cupping and large posterior cupping by length of 100 mm (Figure 1).

Experimentation

Anti-leech assay

First, 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 (Bahmani et al., 2010).

The severity effect of these compounds/drugs based on the time was categorized in five groups as follows (Bahmani et al., 2010):

- 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 of 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 (Bahmani et al., 2010).

Statistical analysis

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

Table 1. Ethno-botanical detail of the *Peganum harmala*.

Scientific name	Traditional treatment (Ghasemipirbalouti, 2009)	Part use	Type of extract	Local name	Secondary compound
<i>Peganum harmala</i> L. (Zygophyllaceae)	Anti-parasite	Fruit	methanol	Esfand	Harmalin, Harmin, Vasicine, Vasicinone

**Figure 1.** The *Limnatis nilotica* leech in water.

RESULTS

Niclosamide and then sulfadimidine were the most and albendazole was the least effective drugs evaluated. The methanolic extract of *P. harmala* L had no effect on *L. nilotica* in 720 min of experiment. The results are demonstrated in Table 2.

Determination phenolic compounds

To standardize the *Peganum harmala* L. studied

in this experiment, the amount of phenolic compounds was measured. The amount of total phenolic compounds in the extract was 98 mg/g equivalent to Gallic acid.

DISCUSSION

In this study, despite of suggestions in Iranian traditional medicine related to indication of *P. harmala* L in leech infestation this experiment showed that *P. harmala* L. (all doses) was ineffective in the killing of *L. nilotica*. The

niclozamide, sulfadimidine, furazolidone and pyvinium, respectively had the most to least effects in killing of leeches and might be used in the treatment of this infection. *P. harmala* L. had not been studied previously against *L. nilotica*, however Bahmani et al. (2010a) studied the effects of tobacco methanolic extract and also some anti-parasite drugs such as mebendazole, metronidazole, triclabendazole, levamisole and succinylcholine. The results of his study showed that tobacco methanolic extract (600 mg) was able to kill the leaches in an average time of 17 ± 6.11 min. The average death time for other

Table 2. The killing time and the severity of drugs effects on leeches.

Treatment	Dose (mg)	Mean \pm SD	Severity
<i>Peganum harmala</i> L.	300	720 \pm 0	-
<i>Peganum harmala</i> L.	600	720 \pm 0	-
<i>Peganum harmala</i> L.	900	720 \pm 0	-
<i>Peganum harmala</i> L.	1200	720 \pm 0	-
<i>Peganum harmala</i> L.	1500	720 \pm 0	-
<i>Peganum harmala</i> L.	1800	720 \pm 0	-
Distilled water	100	720 \pm 0	-
Niclosamide	300	14.77 \pm 3.66	4 ⁺
Albendasole	300	720 \pm 0	-
Pyrrvinium	125	320.44 \pm 300	2 ⁺
Furazolidone	125	137.11 \pm 37.84	2 ⁺
Sulfadimidine	1250	58.33 \pm 22.17	3 ⁺

drugs (triclabendazole, levamisole, and metronidazole) were found to be 118.66, 7 \pm 3.6 and 541.11 \pm 268.49 min, respectively. In another study by Bahmani (2010b), the anti-*L. nilotica* severity effects of closantel, ivermectin, and levamisole were found to be 4⁺, triclabendasole 3⁺, albendasole 2⁺, mebendasole and distilled water had no effect. *Quercus brantii*, *Achillea* spp., *Scrophularia deserti*, *Artemisia kermanensis*, *Artemisia* spp. extracts with dose 600 mg has not have any anti –leech effect, however *Artemisia kermanensis* extract has had dose dependent activity.

In a study by Farkhondeh et al. (2011), levamisole was determined to have a very good effect with 7 min time of killing. The average time of paralysis or death of *L. nilotica* for metronidazole and methanolic extract of *Allium sativum* L. has been reported to be 718.77 \pm 66.3 and 144.55 \pm 57.217 min, respectively.

Bahmani et al. (2011) reported that methanolic extract of *A. sativum* L. exhibited anti leech activities on *L. nilotica* immature form. In this experimental study, the average time of killing of *L. nilotica* (Immature form) for *A. sativum* L. and niclosomide were 68.44 \pm 28.39 and 6.22 \pm 2.94 min, respectively.

Because the proportion of each phytochemical in plants of same species vary in different regions in this study, the *P. harmala* was standardized by measuring the amounts of its phenolic compounds.

P. harmala has been shown to have antiplasmodial (Astulla et al., 2008), antileishmanial (Lala et al., 2004) and anthelmintic (Akhtar et al., 2000) activities. However, it did not show to have anti-leech property.

In the present study, anti-protozoa drugs such as pyrrvinium, furazolidone and sulfadimidine had good effects on *Limnatis nilotica* and might be used per se or in combination with other anti leech drugs.

In conclusion, it is seemed that *P. harmala* L has no killing effect on leeches and this imagination in traditional medicine in Iran about anti-leech effect of *P. harmala* L. is incorrect.

In this study, it was also demonstrated that niclozamide, sulfadimidine, furazolidone and pyrrvinium were effective in killing of *L. nilotica* and might be used alone or as complementary drugs in leech infection.

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