Allium sativum L.: the anti-immature leech (Limnatis nilotica) activity compared to Niclosomide

Mahmoud Bahmani · Javad Abbasi · Ava Mohsenzadegan · Sirous Sadeghian · Majid Gholami Ahangaran

Abstract This study was carried out to determine the effects of methanolic extracts of Allium sativum L. on Limnatis nilotica compared with Niclosomide. In this experimental study in September 2010, a number of leeches (70 in total) from the southern area of Ilam province were prepared, and the effects of methanolic extract of A. sativum L. with Niclosomide as the control drug were compared and distilled water was evaluated as the placebo group which investigated L. nilotica using anti-leech assay. The average time of paralysis and death of L. nilotica for Niclosomide (1,250 mg/kg) and the methanol extract of A. sativum L. (600 μg/ml) were 6.22±2.94 and 68.44±28.39 min, respectively. Distilled water and garlic tablets at a dose of 400 mg were determined as the inert group. In this research, the attraction time of the leeches’ death among different treatments is significant. In this study, it was determined that Niclosomide, with an intensity of 4+, and methanolic extracts of A. sativum L., with an intensity of 3+, have a good anti-leech effect and can be shown to be effective in cases of leech biting, while distilled water was negative.

Keywords Limnatis nilotica · Allium sativum L. · Niclosomide · Paralysis and death

Introduction

Leeches, hermaphroditic, blood-sucking parasites, are rarely reported in humans and animals as a cause of many problems. They vary in color, length, and shape (Ahmadizadeh 2002). 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 tessulatum (Haycox et al. 1995; Vera et al. 2005; Wallis 1988). Leeches mainly inhabit in ponds, lakes, and streams. When water containing young leeches is drunk without necessary caution, leeches enter through the mouth and attach to the upper respiratory system or digestive system mucosa (Pandey et al. 2000). These locations are mostly the nose, nasopharynx, oropharynx, tonsils, esophagus, and occasionally larynx mucosa. Serious complications can include dyspnea, hemoptysis (Pandey et al. 2000) or hematemesis, and vaginal bleeding. Garlic, an indigenous dietary component, belongs to the Liliaceae family and is widely used as a condiment; besides, it is also used in pharmacotherapy against debilitating pathologies because of its antioxidant, anti-hyperglycemic, antimicrobial, antifungal, antithrombotic, antineoplastic, and anti-inflammatory activities (Mayeux et al. 1988; McGrindle et al. 1998; Isaacsohn et al. 1998; Kosciencyl et al. 1999; You et al. 1989; Sabayan et al. 2007; Ledezma and Apitz-Castro 2006; Chung 2006; Sumioka et al. 2006; Su et al. 2006; Zargari 1996). It is an effective drug with no side effects and, as it offers an appropriate cost benefit, the decision
was made to study the anti-leech effects of some herbal and chemical compounds. Considering the effective antiparasitic property of garlic (*Allium sativum* L.), an attempt has been made in the present study to evaluate the efficacy action of the methanolic garlic extract against immature form of leech (*L. nilotica*) in comparision with Niclosomide.

### Materials and methods

#### Study design

In this study, a number of *L. nilotica* immature leeches (70 in total), 30–100 mm in length, were selected from spring water in the southern region of Ilam province (Western Iran). They were dark green in color with rows of green spots on the dorsal surface, yellowish orange and dark green bands on either side, and, as mentioned, a 30–100-mm length—the main signs for the detection of *L. nilotica* immature species. Fresh garlic bulbs (*A. sativum* L./Liliaceae), a popular spice, were preferred.

#### Methanolic garlic extract preparation

The methanolic garlic extract was prepared by adding 1:3 ratios of garlic 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 gathered from the study of the garlic plant has been determined in Table 1. Garlic pills (Garlet) were provided by the Amin Pharmacy Company of Iran. Niclosomide tablets (1,250 mg/kg) (an antiparasite) were investigated as a control and compared with distilled water. These tablets were powdered and diluted in 10 ml distilled water.

#### Anti-leech assay

For the anti-leech assay, the leeches were located individually in a glass container with 600 ml spring water. The extract and drug were then added and their effects observed for 720 min, and time to paralyze, kill, and death of each leech was recorded. The evaluation of a leech’s death 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. 2010a, b).

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

1. **4+:** Paralysis and death of each leech within 1–60 min after addition of the drug,
2. **3+:** Paralysis and death of each leech within 61–120 min after addition of the drug,
3. **2+:** Paralysis and death of each leech within 121–180 min after addition of the drug, and
4. **1+:** Paralysis and death of each leech within 181–240 min after addition of the drug, and
5. **Negative:** Paralysis and death of each leech within 241–720 min after addition of the drug (Bahmani et al. 2010a, b).

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. 2010a, b).

#### Statistical analysis

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

#### Results

The anti-leech activities for treatments on *L. nilotica* are shown in Table 2. The *A. sativum* L. extract showed anti-leech activities with a mean of 68.44 min (3+) in the paralysis and death of the leech (*L. nilotica*). An average time for Niclosomide

| Components                        | Dose    | Time (minute) | Severity |
|----------------------------------|---------|---------------|----------|
| Niclosomide                      | 1,250 (mg/kg) | 6.22±2.94   | 4+       |
| Methanolic garlic extract        | 600 (μg/ml)   | 68.44±28.39 | 3+       |
| Physiological water              | 100 (ml)     | 720±0        | –        |
| Garlet                           | 400 (mg/kg)   | 720±0        | –        |
death was found to be 6.22 (4\(^{+}\)). The result revealed that Garlet and distilled water are not effective in the death of \(L. \text{nilotica}\). Among the treatments tested, Niclosomide and garlic methanol extract showed the best anti-leech activity. The highest effectiveness was found for Niclosomide. Garlet and distilled water demonstrated no anti-leech effect (Table 2).

### Discussion

Garlic (\(A. \text{sativum} \text{ L.}\)) is a popular spice, a remedy for a variety of ailments, and is known for its medicinal uses as an antibiotic, antithrombotic, antineoplastic agent, and anti-inflammatory activities (Ledezma and Apitz-Castro 2006; Chung 2006; Sumioka et al. 2006; Su et al. 2006; Sabayan et al. 2007). Leech infestation can lead to some side effects including anemia, bleeding, subsequent biting infections, ache, itching, inflammation, high sensitivity, and anaphylactic reactions (Ahmadizadeh 2002; El-Awad and Patil 1990; Hadrani et al. 2000; Mirzaei 2005; Raj et al. 2000; Estamble et al. 1992; Madill and Hovingh 2007; Schenkova et al. 2005). In some reports of human, leeches as a foreign body caused acute bleeding of the alimentary system (Kazemi and Bajoghli 2002), hematemesis (Hemmati et al. 2002), or bleeding of the vagina (Yaghmaee 2000), and one case of ophthalmorrhagia caused by leech attachment was reported by Davari (2008). This parasite in the respiratory tract has been reported in animals, and leeches infesting the nasal cavity were reported in one camel in Iraq. Some reports have mentioned that hirudiniasis may cause severe anemia with Hb <5 g/dl (Hemmati et al. 2002).

Garlic, as an effective drug containing no side effects has been suggested for leeches. Hence, the decision was made to study the anti-leech effects of some herbal and chemical compounds. The results of this study showed that garlic methanolic extract (600 \(\mu \text{g/ml}\)) could kill leeches in an average time of 68.44±28.39 min. The average death time for Niclosomide was found to be 6.22±2.94. Garlet and physiological water showed no anti-leech effect. Trade names of garlic components in Iran include Garlet and Garcine pills, Garlic drop, Alicum powder, and the Alum-S pill, which are commonly used to decrease blood cholesterol and triglycerides, cause excretion of esterified acids and alkalis, and decrease blood pressure and anticoagulation (Ghasemi-pirbalouti 2008). Garlic alkaloids have an antimicrobial and antihelminthic nature (Ghasemi-pirbalouti 2008). In the present study, the Garlet pill (without thiosulfinate) and fresh garlic extract were compared. The results revealed that garlic methanolic extract could kill the leech in an average of 68.44 min, but Garlet had no anti-leech effect; therefore, thiosulfinate is a major component for killing leeches. Hashmi Fard (1996) reported that a combination of niclosamide and garlic pill could accelerate the remission of \(T. \text{saginata}\) and \(Hymenolepis \text{nana}\). Soffar and Mokhtar (1991) evaluated the antiparasitic effects of aqueous garlic extract (diluted 1:20) in hirudiniasis and giardiasis. Bahmani et al. (2010a, b) studied the anti-leech effects of tobacco methanolic extract and also some other antiparasitic drugs such as mebendazole, metronidazole, triclabendazole, levamisole, and succinylcholine. The results of this study showed that tobacco methanolic extract (600 \(\mu \text{g/ml}\)) was able to kill the leaches in an average time of 17 min. The average death times for other drugs (triclabendazole, levamisole, niclosamide, and metronidazole) were found to be 118.66, 7, 18.66, and 541.11 min, respectively. In the present study, like previous studies, Niclosomide tablets (1,250 \(\text{mg/kg}\)) revealed anti-leech effects. Bahmani et al. (2010a, b) studied the anti-\(L. \text{nilotica}\) effects of seven anti-parasitic drugs, from which clo- santel, ivermectin, and levamisole were determined at 4\(^{+}\) severity, while triclabendazole (3\(^{+}\)), albendazole (2\(^{+}\)), mebendazole, and distilled water were of negative severity. Lun et al. 1994 revealed antiparasitic effects of diallyl trisulphaide (dasuansu) on human and animal pathogenic protozoa such as \(T. \text{pyriformis}\) sp., \(E. \text{histolytica}\), and \(G. \text{lamblia}\).

### Conclusion

The present study revealed that garlic methanol extract had an anti-leech effect, and therefore, may be used in the treatment of infestation with \(L. \text{nilotica}\) in future.

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