

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

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

Received: 18 September 2011 / Accepted: 29 November 2011 / Published online: 15 December 2011
© The Author(s) 2012. This article is published with open access at Springerlink.com

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 tessulatom* (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 the 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; Kosciely 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

M. Bahmani · M. G. Ahangaran
Young Researchers Club,
Shahrekord Branch,
Islamic Azad University, Iran

J. Abbasi (✉) · A. Mohsenzadegan
General Veterinary Student, Faculty of Veterinary Medicine,
Tehran University,
Tehran, Iran
e-mail: jabbasi@ut.ac.ir

S. Sadeghian
Department of Animal Science,
Faculty of Veterinary Medicine, Tehran University,
Tehran, Iran

Table 1 Scientific name and family name, local name, part used, province, extract type, and traditional treatment

Scientific name and family name	Local name	Part used	Province	Extract type	Traditional treatment (Ghasemi-pirbalouti 2008)
<i>Allium sativum</i> L. (Liliaceae)	Garlic	Bulbs	Tehran	Methanolic extract	Antiparasite

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 comparison 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,
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

Table 2 Distribution of mean \pm SD for the anti-leech activity of methanolic extracts of *Allium sativum* L. and Niclosomide on the immature form of *Limnatis nilotica*

Components Drugs	Dose	Time (minute)	Severity
Niclosomide	1,250 (mg/kg)	6.22 \pm 2.94	4+
Methanolic garlic extract	600 (μ g/ml)	68.44 \pm 28.39	3+
Physiological water	100 (ml)	720 \pm 0	–
Garlet	400 (mg/kg)	720 \pm 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. 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. sativum* 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 µg/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 Alium-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 *Taenia saginata* and *Hymenolepis nana*. Soffar and Mokhtar (1991) evaluated the antiparasitic effects of aqueous garlic extract (diluted 1:20) in hymenolepiasis 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 µg/ml) was able to kill the leeches 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 mg/kg) revealed anti-leech effects. Bahmani et al. (2010a, b) studied the anti-*L. nilotica* effects of seven anti-parasite drugs, from which clorsantel, ivermectin, and levamisole were determined at 4+ severity, while triclabendasole (3+), albendasole (2+), mebendasole, 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 *Trypanosoma* sp., *Entamoeba histolytica*, and *Giardia 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. nilotica* in future.

Open Access This article is distributed under the terms of the Creative Commons Attribution Noncommercial License which permits any noncommercial use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.

References

- Ahmadizadeh A (2002) Leech infestation as a potential cause of hemoptysis in childhood. Arch Otolaryngol Head Neck Surg 128(1):92
- Bahmani M, Avijgan M, Hosseini SR, Gholami Ahangaran M, Sadighara P (2010a) Comparison of anti-*Limnatis nilotica* effect of several anti-nematode, anti-trematodes and anti-cestodes drugs. Vet Res Bull 6(Sup. 1):51–54
- Bahmani M, Avijgan M, Hosseini SR, Qorbani M (2010b) Evaluating the anti-*Limnatis nilotica* effects of tobacco methanol extract compared with succinyl choline and some other anti-parasite drugs. Shahrekord J Med Sci 12(3):53–59
- Chung LY (2006) The antioxidant properties of garlic compounds: allyl cysteine, alliin, allixin, and allyl disulfide. J Med Food 9:205–213
- Davari MA (2008) A case report of ophthalmorrhagia caused by leech attachment. Birjand J Med Sci 14(3):71–75

- Eidi A, Eidi M, Esmaeili E (2006) Antidiabetic effect of garlic (*Allium sativum* L.) in normal and streptozotocin-induced diabetic rats. *Phyto Med* 13:624–629
- El-Awad ME, Patil K (1990) Haemotemesis due to leech infestation. *J Ann Trop Paediatr* 10(1):61–62
- Estamble BB, Knight R, Chung R (1992) Haematemesis and severe anaemia due to a pharyngeal leech (*Myxobdella africana*) in a Kenyan child: a case report. *Trans R Soc Trop Med Hyg* 86(4):458
- Ghasemi-pirbalouti A (2008) Third listen: plants, traditional medicine and ethnoveterinary, medicinal and aromatic plant, 1st edn. Saman-Danesh, Shahrekord, pp 158–190
- Hadrani A, Debry C, Faucon F, Fingerhut A (2000) Hoarseness due to leech ingestion. *J Laryngol Otol* 114(2):145–146
- Hashmi Fard MA (1996) Evaluating and comparison effects of niclosamid together with garlic and low doses of praziquantel on *Hymenolipis nana* in the Neka city. PhD thesis of Pharmacy, Tehran J Med Sci
- Haycox CL, Odland PB, Clotrea MD, Raugi GJ (1995) Indications and complications of medicinal leech therapy. *J Am Acad Dermatol* 33:1053–1055
- Hemmati M, Sat V, Slgee Gh (2002) A case report of vomiting blood with esophageal leech biting. *Kermanshah J Med Sci* 6(4):55–58
- Isaacsohn JL, Moser Stein EA, Dudley DJA, Liskov E et al (1998) Garlic powder and plasma lipids and lipoproteins: a multicenter, randomized, placebo-controlled trial. *Arch Intern Med* 158:1189–1194
- Kazemi A, Bajoghli SH (2002) Two case report alimentary hemorrhagic in children with leech. *J Res Med Sci* 4(8):95–96
- Koscienly J, Klussendorf D, Latza R, Schmitt R, Radtke H, Siegel G (1999) The antiatherosclerotic effect of *Allium sativum*. *Atherosclerosis* 144:237–249
- Ledezma E, Apitz-Castro R (2006) Ajoene the main active compound of garlic (*Allium sativum*): a new antifungal agent. *Rev Iberoam Micol* 23:75–80
- Lun ZR, Burri C, Menzinger M (1994) Antiparasitic effects of diallyl trisulphide (dasuansu) on human and animals pathogenic protozoa (*Trypanosoma* sp. *E. histolytica* & *G. lamblia*) in vitro. *Ann Soc Belge Méd Trop* 74:51–59
- Madill J, Hovingh P (2007) Freshwater leech (Annelida: Hirudinida) distribution in the Canadian Province of Newfoundland and Labrador and adjacent regions: check-list, new records, new pigmentation forms, and Pleistocene refugia. *Magnolia, Zootaxa*, pp 1–21
- Mayeux PR, Agrawal KC, Tou JS, King BT, Lippton HL, Hyman AL et al (1988) The pharmacological effects of allicin a constituent of garlic oil. Agents and pharmacological effects of allicin, a constituent of garlic oil. *Agents Actions* 25:182–190
- McGrindle BW, Helden E, Conner WT (1998) Garlic extract therapy in children with hypercholesterolemia. *Arc Pediatr Adolesc Med* 152:1089–1094
- Mirzaei NI (2005) A case report of epistaxis and haemoptesis at causes leech biting. *J Kurdistan Med Sci* 11:84–87
- Pandey CK, Sharma R, Baronia A, Agarwal A, Singh IV (2000) An unusual case of respiratory distress: live leech in the larynx. *Anesth Analog* 90(5):1227–1228
- Raj SM, Radzi M, Tee Mh (2000) Severe rectal bleeding due to leech bite. *Am J Gastroenterol* 95(6):1067
- Sabayan B, Foroughinia F, Choledry A (2007) A postulated role of garlic organosulfur compounds in prevention of valproic acid hepatotoxicity. *Med Hypotheses* 68:512–514
- Schenkova J, Sychra J, Kubova B (2005) The freshwater leeches (Clitellata: Hirudinida) of the Czech Republic—list of taxa and remarks on rare and endangered species. Department of Zoology and Botany, Faculty of Science, Masaryk University, Kotlarska, 2 (6):11–37
- Soffar SA, Mokhtar GM (1991) Evaluation of antiparasitic effects of aqueous garlic extract in hymenolepiasis and giardiasis. *Parasitology* 21:497–502
- Su CC, Chen GW, Tan TW, Lin JG, Chung JG (2006) Crude extract of garlic induced caspase-3 gene expression leading to apoptosis in human colon cancer cells. *In Vivo* 20:85–90
- Sumioka I, Hayama M, Shimokawa Y, Shiraishi S, Tokunaga A (2006) Lipid-lowering effect of monascus garlic fermented extract (MGFE) in hyperlipidemic subjects. *Hiroshima J Med Sci* 55:59–64
- Vera C, Blu A, Torres M (2005) Leeches, today and yesterday present parasites. *Rev Chilena Infectol* 22:32–37 [in Spanish]
- Wallis RB (1988) Hirudins and the role of thrombin: lessons from leeches. *Trends Pharmacol Sci* 9:425–427
- Yaghmaee M (2000) An abnormal vaginal bleeding at causes of leech. *Zahedan J Res Med Sci* 3(1):41–43
- You WC, Blot WJ, Chang YS, Ershow A, Yang ZT, An Q et al (1989) Allium vegetables and reduced risk of stomach cancer. *J Natl Cancer Inst* 81:162–164
- Zargari A (1996) Medicinal plants. Tehran University Press, Tehran, pp 619–620