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A comparison study on the anti-leech effects of onion (*Allium cepa L*) and ginger (*Zingiber officinale*) with levamisole and triclabendazole

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ABSTRACT

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Anti-leech drugs Ginger Onion Levamisole Triclabendazole **Introduction:** Leech may indwell in mucosa of the pharynx, tonsil, esophagus, nose, nasopharyngeal and rarely in larynx of hosts, however, the effective drugs against this parasite is scarce. This study was aimed to evaluate and compare the anti-leech effect of methanolic extract of onion (*Allium cepa L*) and ginger (*Zingiber officinale*) with levamisole and triclabendazole.

Materials and Methods: In this study, 60 leeches (*Limnatis nilotica*) were collected from south of Ilam. The anti-leech effect of methanolic extract of onion and ginger in comparison with levamisole and triclabendazole drugs (positive controls) were evaluated. Distilled water was used as negative control. Paralysis and death of leeches were recorded in 720 minutes.

Results: Lethal effect of methanolic extract of ginger against *Limnatis nilotica* was equal to levamisole and more than triclabendazole and methanolic extract of onion.

Conclusion: Ginger equal to levamisole has anti-leech activity and its methanolic extract might be used against *Limnatis nilotica*.

Original Article

Implication for health policy/practice/research/medical education: Ginger has anti-leech activity equal to levamisole and its methanolic extract might be used against Limnatis nilotica.

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Introduction

Leeches are blood-sucking hermaphroditic parasites that vary in color and range in length from a few millimeters to half a meter. They are cylindrical or leaf-like in shape, depending on the contraction of their bodies (1). By drinking water that contains leech, it can indwell in mucosa of the tonsil, pharynx, nose, esophagus, nasopharyngeal and rarely in larynx of host and may have serious even lethal complications (2). Aquatic leech infestation occurs rarely, but should be considered as an important cause in the differential diagnosis in endemic areas (3). The clinical symptoms in scorpion sting are typically varied, depending on factors such as scorpion's species, amount of injected venom, season, age and physical conditions of injured patient that may be weak as brief local responses or as dangerous as severely physiological changes that are led to death (4). There is no chemical drug that eliminates leech population without side effects on environmental biology of aquatic animals such as fish (3). World Health Organization suggests investigation on

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control of infection in developing countries (5). In traditional culture of Iranian nomads onion (*Allium cepa L*) used for leech eclipse. Onion has special importance because of vitamins and minerals that it contains and can be used as medicinal plant (6). Onion has been shown to have applications as antimicrobial, antithrombotic, antitumor, hypolipidaemic, antiarthritic and hypoglycemic agents (7). It is used in the treatment and prevention of a number of diseases, including cancer, coronary heart disease, obesity, hypercholesterolemia, diabetes type 2, hypertension, cataract and disturbances of the gastrointestinal tract (e.g. colic pain, flatulent colic and dyspepsia). These activities are related to the thiosulfinates, volatile sulfur compounds, which are also responsible for the pungent of these vegetables (8). Ginger (Zingiber officinale) was found in Asia and Europe many years ago (9). Biological-activity-guided searching for active components showed that zingerone (vanillyl acetone) was the likely active constituent responsible for the antidiarrheal efficacy of ginger (10). Ginger is said to be anti-parasite plant in Iranian herbal medicine (11). The purpose of this study is to evaluate the anti-leech effect of methanolic extract of onion and ginger.

Materials and Methods

Preparation of leeches

Sixty leeches were collected from springs of south of Ilam province. The dark green color surface with rows of green spots on the dorsal surface and yellowish-orange and dark green bands on either side were the main signs for detection of *Limnatis nilotica* species (12). In this study leeches that had the length of 30–100 millimeters was used.

Selection of medicinal plant

Plants were chosen on basis of traditional use of Iranian nomads. Underground root of onion (*Allium cepa L*) and rhizome of ginger (*Zingiber officinale*) were used to preparation of methanolic extract (11).

Preparation of the extracts

Voucher specimen of ginger plant was deposited in Natural Resource Research Center of Tehran province. Rhizomes of ginger were cleaned with water and dried under shade for 5 days until it could be grounded into a powder using an electric grinder. Approximately 200 g was used for extraction. The powder was boiled for 24 h in 500 ml methanolic in a Soxhlet's apparatus. The extract was placed in small test tubes and stored in a refrigerator until required (13).

The anti-parasite drugs

Levamisole and triclabendazole drugs were considered as positive control and distilled water (10 ml) was used as negative control.

The anti-leech assay

The extracts and chemical drugs were then added, their effects were screened for 720 min, and time to paralyze, kill, and death of each leech was recorded. The evaluation of death of a leech was based on immobility after stimulation with a needle. The low average paralyzing and killing time of these compounds reflects anti-leech properties.

The severity of effect of these compounds/drugs based on time was categorized into five groups: (1) $4+\rightarrow$ paralysis and death

of each leech within 1–60 min after addition of the drug, (2) $3+\rightarrow$ paralysis and death of each leech within 61–120 min after addition of drug, (3) $2+\rightarrow$ paralysis and death of each leech within 121–180 min after addition of drug, (4) $1+\rightarrow$ paralysis and death of each leech within 181–240 min after addition of drug, (5) negative \rightarrow paralysis and death of each leech within 241–720 min after addition of drug.

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 (12).

Ethical issues

(1) The research followed the tenets of the Declaration of Helsinki; (2) informed consent was obtained; and (3) the research was approved by the institutional review board.

Statistical analyses

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

Results

As is shown in Table 1, levamisole has intensity of 5.11 ± 1.76 and methanolic extract of onion has intensity of 720 ± 0 min. Ginger has maximum anti-leech effect (33.33 ± 11.40 min).

Table 1. The intensity effect of anti-leech drugs

Treatments	Dose (mg)	Mean±SD	Intensity
Triclabendazole	250	0 ±720	-
Levamisole	50	5.11±1.76	*4
Distilled water	100	720±0	-
Allium cepa L.	600	720±0	-
L. Zingabil officinale	600	33.33±11.40	*4

Discussion

Anti-bacterial and anti-fungal effects of onion are confirmed before (14). In this study methanolic extract of onion had not anti-leech effect. Our results did not confirm the traditional belief of the anti-leech effects of onion. Ginger has suppressed colonic tumor marker formation in rats and induced apoptosis in colon cancer cell lines (15). Among other benefits of the ginger, the results showed that it had anti-leech effects, too. Decrease of the dose of triclabendazole from 500 mm to 250 mm did not cause the death of the leeches, considering this point that triclabendazole is anti-nematode drug and has maximum activity in high doses. According to our results, in comparison with anti-nematodes, levamisole had more effect on the death of the leeches and caused death in 50 mg of its concentration. Note that ginger causes paralysis and death of the leeches in short time, so it can be concluded that ginger is a good natural medicine that can be used as anti-leech in order to decrease the leech pathological effects. In future studies it is recommended that different fractions from ginger be prepared and their antileech effects evaluated. Then, from the effective fraction the chemicals become subject to separation and test to find out the most effective chemical for preparation of a powerful and safe drug.

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Authors' contributions

MB, NVS and MGA prepared the main draft. EB, GH and SB edited the paper.

Conflict of interests

The authors declared no competing interests.

Ethical considerations

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the authors.

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