Global Veterinaria 9 (2): 144-148, 2012
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2012.9.2.63134

Anti-Parasitic Activites of *Zingiber officinale* Methanolic Extract on *Limnatis nilotica*

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Abstract: In the present experimental study, anti-parasitic effect of *Zingiber officinale* on *Limnatis nilotica* leech population was evaluated. After treating the leeches with *Zingiber officinale* (32×10^4 ppm) and the positive controls; Chlor (4×10^6 ppm), Formalin %37 (4×10^3 ppm) and Savlon (4×10^3 ppm) for 30 min, the mean death time of *L. nilotica* was measured by disinfectant assay. The mean death time (M±SD) for *Zingiber officinale* was 24±4.07 min and for Chlor, Savlon and Formalin were 1.62±0.51, 3.37±1.9, 5.12±1.9 min, respectively. The results offer an opportunity for using ginger plant as antiparasitic and disinfectant.

Key words: *Zingiber officinale* · Chlor · Savlon · Formalin · Disinfection · *Limnatis Nilotica*

INTRODUCTION

Using of medicinal plants has abounded because they have fewer side effects and cost of application. The scientific name of ginger plant is *Zingiber officinale* which is cultivated in Asia and has been used in Europe since a very long time ago [1]. The efficacy of ginger is attributed to its aromatic, carminative and absorbent properties [2]. The medicinal properties of ginger include anti-arthritic [3-4], anti-migraine and hypocholesterolaemic [3, 5-7], anti-thrombotic [8, 9], anti-inflammatory [6-10], hypolipidaemic [8, 9, 11, 12], hypocholesterolaemic [11, 13], anti-nausea properties [14, 15], anti-diabetic [12], antipyretic, antimicrobial, antischistosomal, antioxidant, hepatoprotective, diuretic, hypotensive [16] and gastrointestinal prokinetic activities [17]. Leech is an invertebrate of the order Hirudinea. It is Annelid, dark, each over a few inches length with suction cup at its end. This suction cup is used in feeding, movement and attachment to the host [18]. 400 leech species have been identified in zoology and variety of aquatic amphibians [18]. In contaminated water, leech enter mouth, nose and genital system and stick to the lining of the organ painless [19]. Leech biting in human resulted in nasal bleeding [20,21], vaginal bleeding[22], rectal bleeding [23], hematemesis [24], hemoptysis [25] and cause anemia. Inhibitory effect of ginger oil on insect species of Spilosoma was reported [26]. The ginger plant has potent antiparasitic effects [27]. The aim of the current research was evaluation of ginger (*Zingiber officinale*) as a natural disinfectant for killing and clearing leech from water compared with chlor, savlon and formalin as positive control.

MATERIALS AND METHODS

L. Nilotica: In the present study, leeches were selected from spring water in the southern region of Ilam province (west part of Iran) in September 2010. The strong jaws and muscular suckers at the anterior and posterior ends, dark green color surface with rows of green spots on the dorsal surface and yellowish-orange and dark green bands on either side with a 30-100-mm length were the main signs for detection of *L. nilotica* species.

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Table 1: Comparative anti-Leech (Disinfection) activities of *Zingiber officinale* (32×10^4 ppm), Chlor (4×10^6 ppm), Formalin %37 (4000 ppm) and Savlon (4000 ppm)

Xenobiotics	Dosage (ppm)	M±SD (Min)
Zingiber officinale	32×10^4	24±4.07
Chlor	4×10^6	1.62±0.51
Savlon	4×10^3	3.37±1.9
Formalin %37	4×10^3	5.12±1.9
Physiological water	50 ml	30±0

Preparation of Plant Material: 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 methanol in a Soxhlet's apparatus. The extract was placed in small test tubes and stored in a refrigerator until required.

The Anti-parasite (Disinfection) Test: Four leeches were placed in the plastic vessel containing 5 liter of spring water. *Zingiber officinale* (1600mg). Chlor (20g), Formalin (4000ppm) and Savlon (4000 ppm) as the positive controls were used. The experiment was run in three replicates of each group. The number of dead and survivor leeches in each plastic vessel was counted after 30 min [28]. The distilled water was used as negative control. The leeches were considered dead if they did not exhibit any internal or external movement.

Statistical Analysis: The differences between xenobiotics were analyzed using one-way ANOVA statistical method by Sigma State 2.0 software.

RESULTS

The mean death time in minutes (M) M±SD for *Zingiber officinale* methanolic extract was 24±4.07 min, thus ensure that lethality and mortality observed in the bioassay is related to bioactive compounds. The results of the leech lethality test for total groups are presented in Table 1.

DISCUSSION

There is no chemical drug that removes leech population without side effects on aquatic animals such as fish. The natural materials can act more effectively than commercial products. Most of them are less toxic, economical and applicable [29].

According to the study of Farkhondeh *et al*. [30], the average death time of leech for Levamisole was 7 min while for garlic tablet (garlet) were negative. Bahmani *et al*. [28] studied the anti-parasite (Leech) effects of *Nicotiana tabacum* methanolic extract and also some other drugs such as mebendazole, succinyle-choline, metronidazole, triclabendazole, levamisol, niclosamide. It was observed that tobacco methanolic extract (600mg/ml) could kill leaches in the average time of 17 minutes. Average death times for other drugs triclabendazole, levamisole, niclosomide and metronidazole were found 118.66, 7, 18.66 and 541.11 min, respectively [31]. In study by Eftekhari *et al*. [32], the average time of paralysis and the death of *Limnatis nilotica* for Metronidazole, methanolic extract of *Allium sativum L*. and Levamisole was 718.77±66.3 min, 5.11±1.76 min and 144.55±57.217 min, respectively. Bahmani *et al*. [33] reported that methanolic extract of *Allium sativum L*. exhibited anti leech activities on *limnatis nilotica* immature form. They added that the average time of leech death (Immature form) for *Allium sativum L* was 68.44±28.39 min and for niclosomide was 6.22±2.94 min. Bahmani *et al*. [34] evaluated *Quercus brantii*, *Achillea spp*., *Scrophularia deserti*, *Artemisia kermanensis*, *Artemisia spp*. extracts with dose 600 mg which didn't show any anti -*L. nilotica* effect. While, *Artemisia spp*. extract with doses of 1800 and 2400 mg was able to kill leeches with average time of 600±67.8 and 601±37min, respectively. *Artemisia kermanensis* extract with doses of 1800 and 2400 was able to kill leeches in an average time of 635± 67 and 188±61min, respectively. Bahmani *et al*. [28] studied the disinfection effect of *Nicotiana tabacum* extract on *Limnatis nilotica* with LC50 for tobacco 13×10^4 (ppm) and for copper sulfate and ammonium chloride 8×10^4 and 370×10^4 (ppm), respectively. The alcoholic extract of ginger has been studied in human against a specific helminth infestation (*Ascaris lumbricoides*) and was found active [35]. Previous studies have shown that ginger (*Z. officinale*) exhibits anthelmintic activity against *D. immitis* [36], Anisakis larvae [37], *S. mansoni* [38] and gastrointestinal nematodes [39].

Gholami-Ahangaral *et al*. [40] studied effects of the methanolic extract of *Vitis vinifera L.,* niclosamide and ivermectin on *limnatis nilotica* and found that mean death time of leeches treated with niclosamide and ivermectin for mature and immature forms were 15.4 and 11.2 and 10.1 and 11.2 minutes, respectively. The doses of 300 and 600 mg of methanol extract of *V. vinifera* L. against *L. nilotica* mature worm were ineffective but they exhibited death time with 260±63 and 200±50 minutes,

respectively against the immature form of *L. nilotica.* Bahmani *et al*. [41] in an experimental study evaluated effect of the methanolic extract of *Peganum harmala L*. and some of the anti parasite drugs on Limnatis nilotica. 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 extract (300, 600, 900, 1200, 1500 and 1800 mg) were ineffective on leeches in 720 min of experiment. In present study, the same compounds had anti-*L. nilotica* properties. The aromatic principles include zingiberene and bisabolene, while the pungent principles are known as gingerols and shogaols [42].

Bahmani et al showed that ginger has a good effect than anti-parasite drugs (niclosamide and ivermectin) but present study was effect of ginger plant compared to chemical disinfectants. Present study determined stronger disinfection effect of ginger in water polluted with leech for the first time.

In conclusion, the present results suggest that ginger plant extract has good effect on *L. nilotica* and cause less toxic effects than chemical drugs.

ACKNOWLEDGMENT

The authors wish to express their gratitude to the research council Food and Beverages Safety Research Center, Urmia University of Medical Sciences, Urmia, Iran for their financial supports.

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