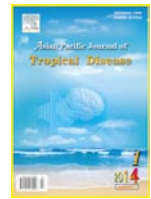




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Comparison of effect of nicotine and levamisole and ivermectin on mortality of leech

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PEER REVIEW

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Comments

This research is a good study in which the authors evaluated anti-leech effects of nicotine on *L. nilotica*. The results are notable and show that native plant had medical beneficity. Details on Page S479

ABSTRACT

Objective: To study the effect of different doses of nicotine on *Limnatis* in comparison with levamisole and ivermectin.

Methods: In this interventional experimental study in July 2012, the amount of 61 mature leeches of *Limnatis nilotica* species were collected and anti-parasitic effects of drug treatments using anti-leech method were assessed. So that, a leech was placed in the dishes with 600 mL spring water and leech's paralysis and death time were recorded accurately for 720 min. A total of 9 replicates were considered for each treatment. Six drug treatments were considered. Pharmacological treatments include nicotine (5, 10 and 20 mg doses), levamisole (10 mg), ivermectin (10 mg) and distilled water. Data were analyzed using the Sigma ASA 2 software and pair *t*-test method with less than 0.05 confidence levels.

Results: The results of present study show that doses of 5, 10 and 20 mg of nicotine, with time average of 2.44±0.52, 1.88±0.78 and 1.55±0.72 min cause to death of leeches. Ivermectin and levamisole cause to death of leeches, averaging 7.44±1.12 and 14.66±5.09 min, respectively. Distilled water treatment has been reported as an ineffective group. Data analysis showed that the group receiving 5 mg nicotine, had minimum time of death and there are statistical differences among all groups (*P*>0.05), but there are not significant differences between treatments receiving 10 mg nicotine, with 5 and 20 mg nicotine treatments.

Conclusions: It appears that nicotine compound as the effective substance of tobacco plant has the strong anti-leech effect on *Limnatis nilotica* species and can be used as leech purposes in the future.

KEYWORDS

Leech, *Limnatis nilotica*, Nicotine, Levamisole, Ivermectine

1. Introduction

Leeches belong to the Annelida category and Hirudinea class. Leeches are blood-sucking hermaphrodite parasites which are attached to their vertebrate host and cause to blood outflowing from the skin by bites. When leech attempting to bite, an anticoagulant substance named

hirudin is secreted that prevents blood clotting and coagulation. Leeches are classified to variety of long, cylindrical, elliptical and flattened forms. Their color may be black, bright or mottled. They have sucker organ at anterior end of body and their length varies between 5 to 45 mm^[1]. Leeches usually enter human body through bathing, swimming or drinking unfiltered and infected waters and

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inhabit in oropharyngeal and nasopharynx mucosa, tonsils, esophagus or nose^[2]. In the tropics, leech bites happen because of walking on infected plants or contaminated waters^[3].

Leech biting could include symptoms such as hemoptysis, nosebleed, respiratory distress, hematemesis, vaginal bleeding and hoarseness^[4–10]. Leech is known as new emerging parasitic zoonosis diseases^[11].

The studies are done on the effects of anti-leech natural and chemical compounds on leeches. In a study, tobacco methanol extract was found has the strong anti-leech effect^[11]. The results of the study showed that garlic tablet even in high dose has not any anti-leech effect on *Limnatis nilotica* (*L. nilotica*) species^[12]. Also a report on the basis of garlic methanol extract effects on the adult leech in *L. nilotica* species has been reported^[13].

In the another study, the effect of hydro alcoholic extracts of oak, yarrow and desert Scrophulariaceae and methanol extracts of sagebrush and wormwood on the *L. nilotica* species is studied and it showed that oak, yarrow and desert Scrophulariaceae hydro alcoholic extracts have no effect on mortality of *L. nilotica*, but methanol extract of wormwood at doses of 1200, 1800 and 2400 mg, with time average of 600 and 601 min (ineffective), respectively and methanol extract of sagebrush at doses of 1200, 1800 and 2400 mg with time average of 635 and 188 min (ineffective) respectively, caused to *L. nilotica* death^[14].

Prevalence of zoonotic infection with leeches because of unfiltered and contaminated waters use is increasing day by day^[15,16]. So use of effective components of plants that their anti-leech effects have been proven is the effective solution to define useful and effective combinations. Antiparasitic effects of tobacco and nicotine have been demonstrated^[17–20].

Nicotine is a nitrogenous organic compound that is found most in plants such as tobacco and in fewer amounts in tomato, potato, eggplant and grin pepper. About 0.3% to 5% of dry plant of tobacco is nicotine which is a poison that affects the nervous system and has been used in many insecticides. Nicotine in low amounts is a stimulator that cause increase in alertness, memory and activity, but in higher amounts lead to increase in heartbeat, blood pressure and loss of appetit.

In pharmacology science, nicotine works as a nicotinic acetylcholine receptor. Nicotine in low amounts cause to increase in these receptors and enhance adrenaline hormone release, which in turn increases the heartbeat, blood pressure, respiration and finally glucose in the blood. Nicotine in higher amounts cause non-polarizes nicotinic acetylcholine receptors, which is one of the reasons of nicotine toxicity, and benefits this features as insecticides^[21–23].

In the previous studies^[11], the effect of methanol extract of tobacco has been proven. As one of the most important anti-parasitic compounds of tobacco plant is nicotine^[17–20]. Therefore in this study combination of nicotine's different doses on the leeches of *L. nilotica* species is evaluated to determine the least and the most effective dose.

2. Materials and methods

In July 2012, 61 immature leeches of *L. nilotica* species were prepared from Gousalo village of Dehloran city in west of Iran, to evaluate anti-leech tests.

In this study, levamisole (Sina Drug, Iran) and ivermectin (Damlran, Iran) drugs were used as a control drug. Also different doses of nicotine combination (Merck, Germany) were used to evaluate the effect of anti-parasitic.

For this purpose, at first in the clear glass dishes with lid (for detailed observation of leeches), a hole with about 0.5 cm diameter was designed to oxygenate and 600 mL of water fountain and a leech were added to the test dish and leeches' paralysis and death precise time were recorded during 720 min accurately, and for each drug treatment, 9 repetition was considered^[20]. Anti-leech effects have been determined based on paralysis and death factors.

Leech death with the sign of lack of movement was determined following leech's body stimulation using the tip of the needle and was reported in terms of minutes during the test and if leeches paralysis and die in shorter time than drug exposure time, treatment is considered more effective^[20]. Effect intensity of treatments based on time was divided into five time ranges including positive four (+4): leech's paralysis and death between 1 to 60 min after adding treatment, positive three (+3): leech's paralysis and death between 61 and 120 min after addition of treatment, positive two (+2): leech's paralysis and death between 121 to 180 min after addition of treatment, positive one (+1): leech's paralysis and death between 181 to 240 min after addition of treatment and negative (-): leech's paralysis and death between 241 to 720 min after treatment addition (even in cases of leech's death in these minutes, it is considered negative in terms of severity of impact). The drugs which caused the death of leeches in 1–60 periods (intensity of +4) were considered as the powerful and effective treatments on leech of *L. nilotica* species^[20].

In this study, six medical treatments were considered. Treatments include nicotine with 5, 10 and 20 mg doses, levamisole (10 mg), ivermectin (10 mg) and distilled water. After 9 repetitions for each treatment, the mean and standard deviation were calculated and reported for each group. To data analysis Sigma ASA 2 software and pair *t*-test analysis method were used. Confidence level for data analysis was considered less than 0.05.

3. Results

Results showed a significant difference between all groups (Table 1) and the group who receive distilled water (as negative control) considering the mean time of leech death ($P < 0.05$). Also between the groups receiving different doses of nicotine, there was only a significant difference ($P = 0.009$) between the groups receiving 5 mg nicotine dose and 20 mg nicotine dose. In the groups receiving 10 mg and 5 mg doses of nicotine and also 20 mg dose, there is no significant difference ($P = 0.139$ and $P = 0.438$, respectively).

Table 1

The results of anti-leech test and leech's death time in different medical treatments.

Treatment	Dose (mg)	Average±Standard deviation	Intensity
Nicotine	5	2.44±0.52 ^d	4 ⁺
Nicotine	10	1.88±0.78 ^d	4 ⁺
Nicotine	20	1.55±0.72 ^d	4 ⁺
Levamisole	10	14.66±5.09 ^b	4 ⁺
Ivermectin	10	7.44±1.12 ^c	4 ⁺
Distilled water	100	720±0 ^a	–

The comparison between nicotine receiving group with other groups show the significant difference between levamisole receiving treatment and different doses of nicotine ($P=0.001$). Average time of leech death following receiving ivermectin, significantly was more than treatment that receiving various doses of nicotine (the comparison level of ivermectin receiving group and nicotine with 20 mg dose was less than 0.001 and was equal 0.004 with other doses of nicotine).

The comparison of death time in two groups of receiving levamisole and ivermectin suggests the significant difference between these two groups ($P=0.003$).

To determine the exact amount of P -value between different groups as pairs, pair t -test was used which the results are specified in Table 2.

Table 2

The exact amount of P -value followed by pair t -test to analyzed groups.

Analyzed groups	P -value amount	Presence/absence of a significant difference
Nicotine 10 vs. Nicotine 20	0.438	Absence of significant difference
Nicotine 10 vs. Nicotine 5	0.139	Absence of significant difference
Nicotine 10 vs. Ivermectin	0.004 (Normality Failed)	Presence of significant difference
Nicotine 10 vs. Levamisole	<0.001	Presence of significant difference
Nicotine 5 vs. Nicotine 20	0.009	Presence of significant difference
Nicotine 5 vs. Ivermectin	0.004 (Normality Failed)	Presence of significant difference
Nicotine 5 vs. Levamisole	<0.001	Presence of significant difference
Nicotine 20 vs. Ivermectin	<0.001	Presence of significant difference
Nicotine 20 vs. Levamisole	<0.001	Presence of significant difference
Levamisole vs. Ivermectin	0.003	Presence of significant difference

4. Discussion

In the present study, anti-leech feature of nicotine in various doses were studied. Nicotine at 5, 10 and 20 mg doses had extremely strong effect. Nicotine at dose of 5 mg with mean of 2.44±0.52 min and effect intensity of 4⁺ was determined as the best treatment.

In study of Bahmani *et al.* in 2011, the anti-leech effect of garlic methanol extract on immature leech of *L. nilotica* species was proved^[18]. In another study of Bahmani *et al.* that examined the effect of anti-*L. nilotica*, 8 of anti-parasitic, anti-nematode, anti-cestode, anti-trematodes drugs, the effect intensity of anti-leech drugs was determined as closantel, levamisole, ivermectin and niclosamid, respectively. With 4⁺ effect intensity that are considered as strong anti-leech drugs. Triclabendazole with effect intensity of 3⁺, albendazole with effect intensity of +2, and mebendazole and metronidazole drugs with effect intensity of negative (–) were determined^[19]. The results of this study are consistent with our study.

In another study that discussed about the anti-*L. nilotica* effect in different doses of Esfand (*Peganum harmala* L.)

methanol extract was found that Esfand methanol extract with doses of 300, 600, 900, 1200 and 1500 mg had no effect on the mature form of *L. nilotica*^[20]. The results of this study are inconsistent with our study. The results of Gholami Ahangarani *et al.* study showed that the methanol extract of grape leaves (*Vitis vinifera* L.) at doses of 300 and 600 mg had no effect on mortality of mature form of *L. nilotica*, while doses of 300 and 600 mg of grape leaves extract was the time average of 260±63 and 200±50 min, respectively, in immature form of *L. nilotica*^[24]. Also the anti-leech effects of ginger extract have been proven. In that study, ginger (*Zingiber officinale*) with the time mean of 33.3±11.4 causes the death of *L. nilotica*^[25]. In another study Gholami Ahangaran *et al.* found that olive methanol extract with mean of 210±24.1 min caused paralysis and death of *L. nilotica*^[26].

Nicotine in higher doses caused non-polarization of nicotinic acetylcholine receptors and this feature used in insecticides^[21–23]. In studies of ethnobotany, different and various effects are recommended for medicinal herbs that can be used for anti-parasitic purposes^[27–30]. Probably anti-leech properties of nicotine are similar to its insecticidal property. Therefore it is recommended that in specialized and pharmacological tests, the mechanism of nicotine effect on leech is tested. Until in the cases of proving its mechanism effectiveness, its pharmacological effects could be used in the future.

Nicotine as a natural secondary compound ingredient of tobacco plant, due to its anti-leech effects in low-dose could be used as an effective anti-*L. nilotica* in the future.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Parasitic infections in animals and humans are one of the medical problems. In recent decades the use of local and medicinal herbs and non-chemical remedies instead of chemical drugs to treat diseases has been regarded. Herbal plants in different areas based on species and amount of active ingredient have different therapeutic effects.

Research frontiers

Studies are being performed in order to determine effects of nicotine (secondary compound) on parasite in experimental.

Related reports

The data of the research about the effect of doses nicotine

on leech is different. This difference is probably due to parasites resistancy.

Innovations & breakthroughs

The effect of nicotine as the tobacco plant active ingredient is first studies and the effect is stronger.

Applications

The results of this study showed that nicotine had good anti-leech effects on *L. nilotica* that it is common in human and animal. So we can test the active compound on other parasites in human and domestic animals.

Peer review

This research is a good study in which the authors evaluated anti-leech effects of nicotine on *L. nilotica*. The results are notable and show that native plant had medical beneficity.

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