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Comparative Assessment of the Anti- *Annelida* (*Limnatis nilotica*) Activity of Nicotine with Niclosamide

Mahmoud Bahmani and Ebrahimkhalil Banihabib

Food and Beverages Safety Research Center,
Urmia University of Medical sciences, Urmia, Iran

Abstract: Nicotine is an alkaloid found in the nightshade Solanacese family of plants, predominantly in the leaves tobacco and in lower concentration in the tomato, eggplant and green pepper. This experimental trial was designed to evaluate the antileech effect of nicotine as well as niclosamide. 32 *Limnatis nilotica* leeched have been prepared, then the experiment was carried out by antileech assay. Results showed that mean death time of leeches in groups treated with nicotine (50 mg) and niclozamide (500 mg) were 1.44±0.52 and 9.77±2.90 min, respectively. It can be concluded that nicotine as a synthetic material from tobacco plant can be used as a strong and effective drug for leech bitting in future.

Key words: Annelida · Antileech · *Limnatis nilotica* · Nicotine · Niclosamide

INTRODUCTION

Leeches are from the Annelida. Leeches are not true worms, but they are described as parasitic worms. Leech belong to the Hirudinea category and they are external parasites for human, domestic and wild animals [1]. Leech have ability up to 900 percent of their body weight for feeding. From those, one that belongs to these 650 species of segmented worms is phylum Annelida is characterized by a small sucker, which contains the mouth, at the anterior end of the body and a large sucker located at the posterior end. All leeches have 34 body segments. The length of the body ranges from a minute to about 20 cm (8 inches) or even longer when the animal stretches. Leeches occur primarily in fresh water and on land. Members of the order Rhynchobdellida occur in the sea as well as in fresh water [2].

Of the leech has been named emerging and newfangled zoonosis parasitic disease [3]. Leeches as a pathogenic parasite cause symptoms including pain, itching, swelling, severe anemia, short-term bleeding, hypersensitivity and even anaphylactic reactions [4, 5].

Studies have shown the potential transmission of important diseases shch as bacterial, viral and parasitic diseases by leeches. Fatal diseases such as AIDS, hepatitis B, syphilis and toxoplasmosis can be transmitted via leeches [6-8].

Bahmani *et al*. [3, 9] claimed that methanol extract of *Nicotina tabacum* has a good antileech effect on *Limnatis nilotica*. The anti parasitic and disinfection effects of the tobacco methanol extract and probably the anti *L. nilotica* effect of the extract are done through the nicotine. Also nicotine is the mian compound of the tobacco plant which has anti leech and anti parasitic activity [3, 9].

Nicotine is an alkaloid which can be found in the nightshade *Solanacese* family of plants, predominantly in the leaves tobacco and in lower concentration in tomato, eggplant and green pepper. They are also found in the leaves of the cocoa plant. Most of the medicinal higher plant extractable organic compounds in sufficient quantities are economocally useful as chemical feed stochs or raw materials for various scientific, technilogical and commercial applications [10].

Nicotine is the main alkaloid of tobacco plant [11]. Nicotine has synonyms of 3-(1-Methyl-2-pyrrolidinyl) pyridine, (S)-(-)-Nicotine and high formula C10H14N [10].

Various studies have shown that nicotine has several pharmacological effects such as increases serum level of the insulin hormone [11]. Laboratory studies showed the inhibition effectsof apoptosis for human lymphocytes cell, immunity of mouse cells as well as the human lung cancer cells [13-15]. Other studies of the neurons in the spinal

Corresponding Author: Mahmoud Bahmani, Food and Beverages Safety Research Center, Urmia University of Medical Sciences, Urmia, Iran. Tel: +98-4412772023.

nerve are protected. Also dose-dependent effects of nicotine in rats have been protecting of feochromocytoma cell [16-18].

Yang *et al*. (2004) claim that nicotine have induced, proliferative and cardiovascular morbidity effects on human umbilical vein endothelial cell proliferation which vessel creating the culture that this effect is dose-dependent [19]. Studies by Villablanca (1998) showed that low doses of nicotine can cause proliferation of endothelial cells, but it in high doses can induce apoptosis [20]. In another study, Zhang *et al*. (2006) determined that nicotine at high concentrations stimulates proliferation and protection against apoptosis in lung cancer cells [21].

Researches has shown that nicotine may disrupt the process of glycolysis [22]. Pharmalogical effects of nicotine on the central and peripheral nervous system were reported [23]. Based on the previous findings, this research was designed to explore the anti *L. nilotica* activities of nicotine in comparison with niclosamide.

MATERIALS AND METHODS

Preparation of Leeches: In august 2012 a number of mature worms (leech) of *L. nilotica* (32 in total) was collected from spring waters in the Gousalou village, Dehloran city, Ilam province of Western part of Iran. 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 *L. nilotica* species [24]. The length of leeches was 20-42 mm.

Chemical Drugs Preparation: In this study, nicotine (Merch, Germany) and niclosamide (Parsdarou, Iran) were used as chemical positive control drugs, thus compared to distilled water. These tablets were powdered firstly and then diluted in 10 ml distilled water and added to glass test.

Anti-Leech Assay: For the anti-leech assay, the method of Bahmani *et al*. [24] was used. Frisrtly, the leeches were located individually in a glass container with 600 ml spring water.

The compounds/drugs were then added and leeches were screened for 720 min and the time of paralysis and death of each leech was recorded. The severity of antileech 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 drug, (3) 2+→ paralysis and death of each leech within 121–180 min after addition of drug, (4) 1+→ paralysis and death of each leech within 181–240 min after addition of drug, (5) negative → paralysis and death of each leech within 241–720 min after addition of drug [24].

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 [24].

Statistical Analysis: Death time was recorded for each of the leeches and entered into Excel files. For data, the differences between the control and treatement groups were analyzed using Sigma State 2.0 software program and one-way ANOVA test.

RESULTS

The nicotine showed anti *L. nilotica* affect with 4+ severity. The result showed that niclosamide had good efficacy on leeches while distilled water with negative severity (-), have no effect on *L. nilotica.* The details of the results were given in Table 1.

There are a significant differences between treatment groups ($p<0.001$). Normality Failed with median of 1, 10 and 720 for nicotin, nicloz and water respectively.

DISCUSSION

In this study the effects of nicotine on *L. nilotica* was investigated. The results showed that nicotine causes death of leeches in a short time.

Based on the studies, many effects of nicotine depend on the ability of the drug to interact with various neurotransmitter systems [25]. The drug with effects on the central and peripheral nervous system makes many pharmacological effects [23].

In this study nicotine could have high intensity to stimulate the movements of the worms and eventually became the cause of paralysis and death of leeches.

Table 1: Effects of nicotine and niclosamide treatments on *L. nilotica*

Components/Drugs	Dose (mg)	Mean death time± SD (Min)	Severity
Nicotine	50	1.44±0.52a	4+
Physiological water	100	720±0c	-
Niclosamide	500	9.77±2.90b	4+

Many substances are obtained from nicotine which can have a role in controlling the disorders or diseases, for example nicotine gum, troche, sublingual tablets, label nicotine patch, nicotine inhaler, nasal spray and antidepressants that includes varenicilin that used for smoking cessation; obtained from nicotine [26-28]. Nicotine is also used in the treatment of diseases such as Parkinson, Alzheimer and ulcerative colitis [29].

Farkhondeh *et al*. [30] performed a study which shows that garlic tablets can't cause fatality of leeches. In Farkhondeh's study, the thiosulfinic components of garlic are named as active and main anti *L. nilotica*.. In other study, Bahmani *et al* .[31] showed the garlic methanol extract results in the death of immature leechese and is anti *L. nilotica*. The study by Eftekhari *et al*. [32]. releaved that the methanol extract of garlic with the strong effect on adult worms of *L. nilotica* causes the death of leeches.

In a perevious study by Bahmani [35], tobacco methanol extract with a dose of 600 mg has killed the leeches in an average of 17±6 mins. In this study, nicotine (50 mg) has killed the leeches with death time of 2 mins that is about 1/12 of the time that they were killed by tobacco methanol extract (17±6 min) and concluding that nicotine as the main active ingredient of tobacco plant.

Bahmani *et al*. [33] have evaluated the anti-*Limnatis nilotica* effects of anti-parasite drugs and reported that closantel, ivermectin, niclosamide and levamisole have a killing effect with 4+ severity, while triclabendasole and albendasole have 3+ and 2+, respectively.

In other study by Gholami-Ahangaral *et al*. [34], the effects of *Vitis vinifera* L methanolic extract, niclosamide and ivermectin on *L. nilotica* were evaluated and they found that grape has anti *L. nilotica* effect.

Two studies have showed that *Zingiber officinale L.* has anti leech and disinfactant effects on *L. nilotica* [36, 37].

It appears that the cellular effects of nicotine are done through its nicotine acetylcholine receptors [38]. In addition, these receptors are widely distributed in nerve cells and neuromuscular connections [38, 39].

From the findings of this study , probably it can be concluded that nicotine killed the leeches in a short time of exposure. Based on its mechanism, it is recommended that the efficacy of nicotine be evaluated on other leech pathogens. Also in another study the mechanism of fatality of the leeches via nicotine can be discovered.

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