

# The anti-parasitic effects of *Nicotina tabacum* on leeches

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Received: 26 June 2011 / Accepted: 5 January 2012 / Published online: 27 January 2012  
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**Abstract** In this study, we evaluated the potential use of *Nicotina tabacum* in controlling leech populations. After treating the leeches with different concentrations of *N. tabacum* for 30 min, the LC<sub>50</sub> values were analysed. Copper sulphate and ammonium chloride were used as positive controls. The anti-parasitic effect of *N. tabacum* was compared with positive controls. Our data showed that the LD<sub>50</sub> values for *N. tabacum* were  $13 \times 10^4$  ppm which was considerable compared with positive control. Our finding offers an opportunity for using the plant for anti-parasite purposes.

**Keywords** *Nicotina tabacum* · Leech · *Limnatis nilotica*, anti-parasite

## Introduction

Leeches are small invertebrate animals from the phylum annelids and subclass Hirudinea; they vary in colour and length from half an inch to 10 in. in length (Sasaki and Tani 1997). There are freshwater, terrestrial and marine varieties.

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Leeches live in lakes, ponds, marshes, springs and slow streams (Sasaki and Tani 1997). So far 650 species of leeches have been recognized; 50 species feed on mammalian blood, highlighting the importance of the parasitic leeches (Cook 1996). They can cause serious problems in animals such as anaemia, haemorrhage, pain, itching and anaphylaxis reactions. Human leech infestation presents with different clinical symptom such as haemoptysis, haematemesis and vaginal bleeding (El-Awad and Patil 1990). Leech infestation primarily occurs in tropical areas, such as Mediterranean countries, Africa and Asia (Uygur et al. 2003). Leech infestation has also been reported from different regions of Iran.

Chemical drugs are commonly used against several leeches. Safe environmental chemicals can effectively reduce leech populations (Sasaki and Tani 1997). Tobacco (*Nicotina tabacum*) is an agricultural product processed from the leaves of plants in the genus *Nicotina*. Tobacco contains nicotine, a highly toxic substance which both kills and repels different pests on flowering plants, fruits and vegetables and has been used as a natural organic pesticide for hundreds of years. The aim of this study was to investigate tobacco as a natural anti-parasitic drug for removing leeches from water compared with positive control (copper sulphate and ammonium chloride).

## Materials and methods

### Taxonomy of leeches

The leeches were gathered from the Ilam province (the west of Iran). The species of leech was determined by Dr. Behnam Meshgi. Voucher specimen of this leech was deposited in the parasitology department, Veterinary Medicine Faculty of Tehran University.

## Plant material

*N. tabacum* leaves were also collected from Ilam and identified by Dr Ayob Pirani. Voucher specimen of the plant was deposited in natural resource research centre of the province. The leaves were separated, air-dried in the shade and powdered. Fifty grams of powder was soaked in 300 ml of methanol for 2 days. The solvents were then evaporated in a rotary evaporator. The extract was placed in an oven at 50°C for 2 days to prepare the dry powder.

## Evaluation of the anti-parasitic effects of the drugs

The leeches were placed in the plastic vessels containing water. The plant extract with different concentrations ( $6 \times 10^4$ ,  $12 \times 10^4$  and  $18 \times 10^4$  ppm) was added to the vessel. The experiment was run in three replicates of each concentration. The number of living and dead leeches in each tube was counted after 30 min. In the positive control group, different concentrations of copper sulphate ( $5 \times 10^4$ ,  $10 \times 10^4$  and  $20 \times 10^4$  ppm) and  $\text{NH}_4\text{Cl}$  ( $10 \times 10^4$ ,  $50 \times 10^4$  and  $200 \times 10^4$  ppm) were chosen. Distilled water was used as negative control. The leeches were considered dead if they did not exhibit any internal or external movement.

## Results

After 30 min of treatment, the number of dead and alive leeches was counted. The results of the leech lethality test are presented in Table 1. The  $\text{LC}_{50}$  values (the concentration at which half the leeches died) for *N. tabacum* was  $13 \times 10^4$  ppm, and copper sulphate and ammonium chloride were  $8 \times 10^4$  and  $370 \times 10^4$  ppm, respectively. To ensure that the mortality observed in the bioassay was only related to bioactive compounds, distilled water was considered as negative control. The negative control had no effect on the leeches.

## Discussion

Aquatic leech infestation occurs rarely, but should be considered as an important cause in the differential diagnosis in endemic areas (Cook 1996). For the first time,

the anti-parasitic effects of *N. tabacum* against leeches were investigated.

There is no chemical drug that eliminates leech populations without side effects on the environmental biology of aquatic animals such as fish. Natural materials can be more effective and more powerful than commercial products. The majority is less toxic, more economical, possesses no ethical problem and is applicable (Sasaki and Tani 1997). *N. tabacum* has been cultured worldwide; therefore, it is easily available. According to our finding, the  $\text{LC}_{50}$  of tobacco extract was significantly lower than ammonium chloride. Although the lethality of copper sulphate was higher than tobacco extract, the effects of the plant extract did not show an activity as strong as the copper sulphate. However, using copper sulphate in livestock as a fungicide has an additional factor in that it is toxic. Copper sulphate is a common chemical that is used as an algicide, parasiticide and molluscicide and is toxic to fish. It induces a significant increase in DNA damage in *Allium cepa* root cells (Yildiz et al. 2009) and causes a loss of electrolytes (Perschbacher 2005). Moreover, copper sulphate is corrosive to metal containers. Therefore, with respect to the side effects, even at low concentration, the application of copper sulphate to control leech populations is not recommended.

The tobacco plant has been used as an antispasmodic, diuretic, emetic, expectorant, irritant and sedative and sialagogues drug. The nicotinic composition of the plant has pesticide activities. Nicotine interacts with nicotinic acetylcholine receptors leading to muscle weakness (Narahashi et al. 2000). Both vertebrate and invertebrate species have nicotinic receptors with many similar structural and functional properties, but they exhibit some different physiological and pharmacological characterization. The affinity of nicotine to vertebrate nicotinic receptors is lower than insect nicotinic receptors (Ballivet et al. 1996). Furthermore, the carcinogenic and mutagenic properties of nicotine have not been proven. The risk of teratogenic defects caused by nicotine is very small or nonexistent (Alexander and Glassman 1999). It is suggested that *N. tabacum* extract is effective in controlling leech population.

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**Table 1** A comparison  $\text{LC}_{50}$  (in parts per million) between xenobiotics

Xenobiotics	$\text{LC}_{50}$ (ppm)
<i>N. tabacum</i>	$13 \times 10^4$
Copper sulphate	$8 \times 10^4$
Ammonium chloride	$370 \times 10^4$
Distilled water	–

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