



In-vitro* effect of some plant extracts on Buffalo leech, *Hirudinaria manillensis

JOKEN BAM¹, S ISLAM², D BHATTACHARYA³, D K DEKA⁴, D J KALITA⁵, R K SHARMA⁶,
PALLABI PATHAK⁷, S MAITI⁸, A K BERA⁹ and S M DEB¹⁰

Assam Agricultural University, Khanapara, Asom 781 022 India

Received: 3 December 2015; Accepted: 2 February 2016

ABSTRACT

The study was conducted to find out the anti-leech effect of 5 indigenously used plant species on *Hirudinaria manillensis*. Aqueous and methanolic extracts of *Nicotiana* spp., *Zanthoxylum alatum*, *Solanum khasianum*, *Cucumis sativus* and *Lasia spinosa* were prepared. The experiment was designed to evaluate 3 concentrations of aqueous (@5,000, 2,500 and 1,000 µg/ml) and methanolic extracts (@ 500, 100 and 50 µg/ml) of all the plants along with levamisole (83 µg/ml) as standard and distilled water as control. The leeches were treated with the determined concentration of extract/standard and the death time (min) was recorded up to 720 min. The aqueous extract of *Nicotiana* spp. and *Z. alatum* showed best anti-leech effect with mean death time ranging from 2.11±0.11 to 5.33±0.47 min and comparable result with the standard at 83 µg/ml concentration. The methanolic extracts of *Z. alatum* at all the concentrations and *Nicotiana* spp. and *S. khasianum* at 500 µg/ml showed no significantly different effect with levamisole standard. The methanolic extract of *Z. alatum* exhibited best hirudinicidal effect (10.56±2.69 min) against *H. manillensis*. Aqueous extract of *S. khasianum* and both aqueous and methanolic extracts *C. sativus* and *L. spinosa* showed no killing effect on *H. manillensis* at all the concentrations. It was concluded that among the 5 plant species, *Nicotiana* spp., *Z. alatum* and *S. khasianum* were effective against *H. manillensis*.

Key words: Anti-leech, Aqueous extract, Death time, Leech, Methanolic extract, Repellent

Leeches are segmented hermaphroditic worms under the phylum Annelida and order Hirudinea widely distributed all over the world, except the polar continents. Leeches attach themselves to various animal species through their suckers, feed on blood and drop off after having completely engorged (Soulsby 1986). Apart from blood loss and discomfort caused by leech bite, they also transmit many blood borne pathogens. Many disease causing viruses, bacteria and protozoan parasites were recorded in African leeches, so leeches pose a considerable threat to their hosts (Mehlhorn 2001).

Hirudinaria manillensis is commonly known as the buffalo leech. This leech may have a potential role in transmission of *Trypanosoma evansi* (Desquesnes *et al.* 2013). The indigenous people of many tribal areas use herbs

as medicine and insect repellents. Not only in India, but the ethnic groups of Lao People's Democratic Republic also use botanical repellents and pesticides to keep leeches at bay (de Boer *et al.* 2010). As far as Indian scenario is concerned, use of indigenous traditional knowledge as leech repellent and to control the after-effects of infestation has been used mainly by the tribes of north-eastern states (Choudhury *et al.* 2007, Bam *et al.* 2015), Andaman and Nicobar Islands (Shareif 2007), hilly tracts of Tamil Nadu (Ayyanar and Ignachimuthu 2010) and *Gujjar* community of Sub-Himalayan tracts of Uttarakhand (Gaur *et al.* 2010). The present study was designed to evaluate the hirudinicidal effect of 5 such plants reported to possess anti leech effect in the Indigenous Traditional Knowledge (ITK) on *Hirudinaria manillensis*.

MATERIALS AND METHODS

Collection and processing of plant materials: Fresh fruits of *Solanum khasianum*, peelings of *Cucumis sativus* fruit, fruits of *Zanthoxylum alatum* and tender stems of *Lasia spinosa* were collected in clean plastic bags. Tobacco leaves were purchased from local market, Guwahati. The plant samples were dried in the shade away from direct sunlight in a well ventilated area. Individual dried materials were pulverized in feed grinder and stored in airtight containers.

Preparation of crude plant extracts: Crude extracts of plant materials were prepared in water and methanol. Plant

Present address: ¹Scientist (joken.nrcy@gmail.com), ^{2,4}Professor (isaidul@gmail.com, dilipkumar.deka@rediffmail.com), ³Principal Scientist (debasis63@gmail.com), ⁵Associate Professor (djkalita@rediffmail.com), Department of Veterinary Biochemistry. ⁶Professor (dr.sharmark@rediffmail.com), Department of Microbiology, College of Veterinary Science. ⁷PhD Scholar (pallabipathak7@gmail.com), Department of Parasitology. ⁸Scientist (sanjit.ndri@gmail.com), ICAR-National Dairy Research Institute, Karnal, Haryana. ⁹Senior Scientist (asitmed2000@yahoo.com). ¹⁰Director (sm_deb@yahoo.com), ICAR-National Research Centre on Yak, Dirang, Arunachal Pradesh.

powder (500 g) was dissolved in 3,000 ml of distilled water by continuous stirring with glass rod. It was allowed to stand for 48 h in a cool dry place. After 48 h, the materials were filtered through 4 folds of fine muslin cloths. For preparation of methanolic extract, plant powder (4 g) was taken in a thimble and extracted at 40°C in Soxhlet apparatus using methanol until the color of plant material became clear. The extracts from both the methods were filtered through Whatmann filter paper No.4, poured into large petridishes and dried in hot air oven at a temperature of 40°C. After complete evaporation, thin layer of extract on the surface of the petridish was scrapped out and preserved in air tight containers at 4°C until use (Rawlins 1986).

Standard drug: Dicaris adult tablets containing 50 mg of Levamisole were used as standard drug to compare the leech repellent activity with that of plant extracts.

Methodology: Uniform sized *Hirudinaria manillensis* leeches (300) were collected from the wetlands and paddy fields of Asom and maintained in laboratory. The leeches were identified using the keys of Harding and Moore (1927). Three concentrations each of aqueous (1,000 µg/ml, 2,500 µg/ml and 5,000 µg/ml) and methanolic extract (50 µg/ml, 100 µg/ml and 500 µg/ml) were selected for evaluation. For each concentration, 9 beakers containing 200 ml of required concentration of extracts were prepared in distilled water. One leech was put into each beaker and the time of mortality was recorded upto 720 min (Ahangaran-Gholami 2012) using a stop watch. Leeches showing no response upon pricking with a needle were considered as dead. For control, 15 leeches were kept in beakers containing 200 ml of distilled water and observed for 720 min. Similarly for standard, 15 leeches were introduced into 15 individual beakers containing 200 ml Levamisole (83 µg/ml) dissolved in distilled water and time of mortality was recorded.

Statistical analysis: The time of mortality data obtained were subjected to one way ANOVA using SPSS Version 20 and to test the difference of mean among the plants, Duncan's Multiple Range Test (DMRT) was done.

RESULTS AND DISCUSSION

The aqueous extract of *Nicotiana* spp. and *Zanthoxylum* spp. showed most effective and quick hirudinicidal activity

at 2,500 and 5,000 µg/ml i.e 2.11±0.11 to 5.33±0.47 min in comparison to the Levamisole @ 83µg/ml (Table 1). The hirudinicidal activity of *Nicotiana* spp. at 1,000 µg/ml (16.00±5.45 min) was comparable with that of standard (16.13±1.38 min) drug and same dose of *Zanthoxylum* spp. (16.89±1.48) but not significantly different from higher doses of both *Nicotiana* spp. and *Zanthoxylum* spp. The mean death time (min) of Nicotine pure compound on *Limnatis nilotica* was 1.44±0.52 @ 83.3 µg/ml (Bahmani and Banihabib 2013). *Limnatis nilotica* is a notorious leech found in the natural springs in the country sides of Iran infecting many livestock species and poultry (Mozaffari *et al.* 2011, Ghazvinian *et al.* 2014, Bahmani *et al.* 2015).

The aqueous extracts of *S. khasianum*, *C. sativus* and *L. spinosa* did not show any effect on leeches during the study. Many species of the genus *Solanum*, i.e., *S. erianthum*, *S. melongena*, *S. khasianum* or *S. viarum* and *S. nigrum* are used traditionally by the indigenous people as a protection against leeches in animals and man (Ayyanar and Ignachimuthu 2010, de Boer *et al.* 2010, Bam *et al.* 2015). Kalita *et al.* (2004) reported a common practice of use of a paste made of leaves of bitter gourd along with peels of *C. sativus* for control of leeches by fish farmers of Asom.

The result of mean death time (min) of methanolic extracts on *H. manillensis* showed significant differences between different doses and different plant species. The shortest mean death time i.e., 10.56±2.69 was shown by *Z. alatum* at 500 µg/ml. The mean death time of methanolic extract of *Nicotiana* spp. (@ 500 µg/ml), *Z. alatum* (500 µg/ml, 100 µg/ml and 50 µg/ml) and *S. khasianum* (@ 500 µg/ml) was comparable with Levamisole. The *Nicotiana* spp. @ 500 µg/ml and 100 µg/ml showed significant effect compared to the lower dose i.e @ 50 µg/ml. In a similar study, Bahmani *et al.* (2010) studied the effect of methanolic extract of *N. tabacum* @ 50 µg/ml on *L. nilotica* and mean mortality time of 17 min was recorded. All the 3 concentrations of *Z. alatum* (500 µg/ml, 100 µg/ml and 50 µg/ml) in the present study showed comparable result with Levamisole and there was no significant difference between the different doses. On the other hand, all the 3 doses of methanolic extract of *S. khasianum* showed significant difference between doses ranging from

Table 1. Death time (min) of *H. manillensis* on treatment with plant extracts (mean±SE)

Conc. (µg/ml)	<i>Nicotiana</i>	<i>Z. alatum</i>	<i>S. khasianum</i>	<i>C. sativus</i>	<i>L. spinosa</i>
<i>Aqueous extract</i>					
5000	02.11±0.11 ^a	03.00±0.33 ^a	720.00±00 ^h	720.00±00 ^h	720.00±00 ^h
2500	02.67±0.24 ^a	05.33±0.47 ^{ab}	720.00±00 ^h	720.00±00 ^h	720.00±00 ^h
1000	16.00±5.45 ^{abcd}	16.89±1.48 ^{abcd}	720.00±00 ^h	720.00±00 ^h	720.00±00 ^h
<i>Methanolic extract</i>					
500	19.33±4.31 ^{abcd}	10.56±2.69 ^{abc}	24.89±2.34 ^{cde}	720.00±00 ^h	720.00±00 ^h
100	29.44±3.16 ^{de}	12.56±1.37 ^{abc}	79.67±12.51 ^f	720.00±00 ^h	720.00±00 ^h
50	35.33±4.24 ^e	20.56±2.30 ^{abcd}	113.67±20.99 ^g	720.00±00 ^h	720.00±00 ^h
Standard (Levamisole, 83 µg/ml)	16.13±1.38 ^{abc}				
Control (Distilled water)	720.00±00 ^h				

Significant at P<0.05.

24.89±2.34 to 113.67±20.99 min. Irrespective of the method of extraction and dose, both *C. sativus* and *L. spinosa* did not show any effect on *H. manillensis*. On the basis of the present findings, it may be concluded that *Nicotiana* spp., *Z. alatum* and *S. khasianum* used as ITK by several indigenous people are effective against leeches.

ACKNOWLEDGEMENT

The authors are thankful to the Head of the Department of Parasitology and Dean, Faculty of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam for the support and encouragement. We acknowledge Department of Biotechnology for the financial support for the study through DBT Twinning project.

REFERENCES

- Ahangaran-Gholami M, Bahmani M and Zia-Jahromi N. 2012. Comparative and evaluation of anti-leech (*Limnatis nilotica*) effect of olive (*Olea europaea* L.) with Levamisol and Tiabendazole. *Asian Pacific Journal of Tropical Diseases* 101–3.
- Ayyanar M and Ignacimuthu S. 2010. Plants used for non-medicinal purposes by the tribal people in Kalakad Mundanthurai Tiger Reserve, Southern India. *Indian Journal of Traditional Knowledge* 9(3): 515–18.
- Bahmani M and Banihabib B. 2013. Comparative assessment of the anti- Annelida (*Limnatis nilotica*) activity of nicotine with niclosamide. *Global Veterinaria* 10(2): 153–57.
- Bahmani M, Gholami A M, Ghotbian F, Hassanzadazar H, Karamati S A, Sepahvand R and Darabi A. 2015. Internal contamination with leech in a turkey. *Asian Pacific Journal of Tropical Disease* 5(2): 158–60.
- Bahmani M, Hosseini S R, Avijgan M and Qorbani M. 2010. Evaluating the anti-leech effect of tobacco methanolic extract compared with succinyl choline and some other anti-parasitic drugs. *Journal of Shahrekord University of Medical Sciences* 12(3): 53–59.
- Bam J, Rai S, Bhattacharya D, Maiti S, Islam S, Pathak P, Bera A K and Deb S M. 2015. Indigenous curative and prophylactic traditional practices used against haematophagous leeches in Arunachal Pradesh and Sikkim. *Indian Journal of Traditional Knowledge* 14(3): 493–97.
- Choudhury B I, Khan M L, Arunachalam A and Arunachalam K. 2007. *Gymnocladus assamicus* Kanjilal ex P. C. Kanjilal fruito: A soap substitute. *Nature Product Radianc* 6(5): 427–29.
- de Boer H J, Vongsombath C, Palsson K, Bjork L and Jaenson T G T. 2010. Botanical repellents and pesticides traditionally used against hematophagous invertebrates in Lao People's Democratic Republic: A comparative study of plants used in 66 villages. *Journal Medical Entomology* 47(3): 400–14.
- Desquesnes M, Dargantes A, Lai D, Lun Z, Holzmuller P and Jittapalapong S. 2013. *Trypanosoma evansi* and Surra: A review and perspectives on transmission, epidemiology and control, impact, and zoonotic aspects. *BioMed Research International* 2013: 20. (<http://dx.doi.org/10.1155/2013/321237>).
- Gaur R D, Sharma J and Painuli R M. 2010. Plants used in traditional healthcare of livestock by Gujjar community of sub-Himalayan tracts, Uttarakhand, India. *Indian Journal of Natural Products and Resources* 1(2): 243–48.
- Ghazvinian K, Rassouli M, Darvishi M M and Massoum M A. 2014. Heavy leech infestation in imported camels to Iran. *Journal of Camelid Science* 7:67–70.
- Harding W A and Moore J P. 1927. *The Fauna of British India, including Ceylon and Burma: Hirudinea*. Taylor & Francis, London.
- Kalita B, Choudhury M and Ojha S N. 2004. Indigenous technical knowledge on pond construction and maintenance, fish seed transportation, and fish health management in Asom hills. *Indian Journal of Traditional Knowledge* 3(2): 192–97.
- Mehlhorn H. 2001. *Encyclopaedic Reference of Parasitology Biology: Structure: Function*. 2nd edn. Springer.
- Mozaffari A A, Ebrahimi P R and Imani M. 2011. An unusual case of subglossal Hirudiniasis in an Iranian cross-breed goat-kid. *Iranian Journal of Veterinary Surgery* 6(1,2): 65–68.
- Rawlins E A. 1986. *Textbook of Pharmaceuticals*. 8th edn, CBS Publication, London.
- Sharief M U. 2007. Plants folk medicine of Negrito tribes of Bay Islands. *Indian Journal of Traditional Knowledge* 6(3): 468–76.
- Soulsby E J L. 1986. *Helminths, Arthropods and Protozoa of Domesticated Animals*. ELBS. 7th edn. Bailliere and Tindall. London.