SHORT COMMUNICATION

Biological activities of *Peristrophe bivalvis* extracts: promising potential for anti-snake venoms against *Naja kaouthia* and *Trimeresurus albolabris* venoms

Jatuporn Phaopongthai\textsuperscript{a*}, Jureeporn Noiphrom\textsuperscript{b}, Supat Phaopongthai\textsuperscript{a}, Narumol Pakmanee\textsuperscript{b} and Jirapast Sichaem\textsuperscript{c}

\textsuperscript{a}Faculty of Science and Technology, Rajamangala University of Technology Thanyaburi, Thanyaburi, Pathum Thani, 12110, Thailand; \textsuperscript{b}Research and Development Department, Queen Saovabha Memorial Institute, Pathumwan, Bangkok 10330, Thailand; \textsuperscript{c}Natural Products Research Unit, Department of Chemistry, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand

(Received 8 February 2015; final version received 2 April 2015)

This study evaluates the \textit{in vitro} anti-snake venom potential of *Peristrophe bivalvis* (PB) extracts against *Naja kaouthia* (NK) and *Trimeresurus albolabris* (TA) venoms, including inhibition of cytotoxic effects and enzymatic activities, and the binding-precipitation of extracts and venom proteins analysis. In addition, the antioxidant, cytotoxic and \textit{in vivo} acute oral toxic activities of PB extracts are also reported. The \textit{in vitro} cytotoxic and enzymatic analysis reveals that the ethanol extracts of stems and leaves of PB showed good anti-snake venom activity against NK and TA venoms. In addition, the antioxidant result indicated that only the ethanol extract of leaves exhibited weak DPPH radical-scavenging activity. The ethanol whole-plant extract of PB also showed no cytotoxicity against four cell lines. Moreover, the \textit{in vivo} acute oral toxicity result of the ethanol whole-plant extract showed that all treated rats did not exhibit abnormal toxic signs or deaths.

\textbf{Keywords:} *Peristrophe bivalvis*; anti-snake venom activity; cytotoxicity; antioxidant activity; acute oral toxicity

1. Introduction

*Peristrophe bivalvis* (L.) Merr. (Acanthaceae) is a wild-growing plant that is sometimes cultivated by ethnic minorities for dyeing foods, such as sticky rice, and other dishes (Thuy et al. 2012). It has been used in traditional medicine for the treatment of pulmonary tuberculosis, haemoptysis, bronchitis, congestion and wrench (sprain) (Trinh et al. 2003). The presence of aliphatics, 14-
methyltritriacont-14-en-15-ol and 35-hydroxynonatriacontanal (Singh et al. 2000), and two phenoxazine alkaloids, peristrophine and perisbivalvine A (Thuy et al. 2012) have been reported as some of the phytochemicals present in this plant. In Thailand, this plant was also putatively considered for an inhibitor of snake venom in Thai traditional herbal recipes. The authors had recommended the rice whisky extract from PB leaves used as a Thai traditional medicine for the emergency treatment of snakebites. However, the efficacy of the plant as an anti-snake venom was not supported by scientific evaluation. Therefore, this investigation studied the actions of PB extracts [ethanol extract of stems (Extr. 1), a whole plant (Extr. 2) and leaves (Extr. 3); 40% extract of leaves (Extr. 4) and boiled water extract of leaves (Extr. 5)] on anti-snake venom, including inhibition of cytotoxic effect and enzymatic (PLA$_2$ and protease) activities, and analysis of the binding-precipitation with venom proteins of Naja kaouthia (NK) and Trimeresurus albolabris (TA) venoms, as the representative of neurotoxic and haematotoxic snake venoms which induce local damage by in vitro methods. Moreover, their antioxidant (DPPH), cytotoxicity (Vero, Caco2, HepG2 and HEp-2 cells) and in vivo acute oral toxic activities were also disclosed in this study.

2. Results and discussion

2.1. Inhibitory activity of extracts against NK and TA cytotoxicity

The results indicated that all extracts (Extr. 1–5) of PB showed good activity against snake venoms. Although the ethanol leaf extract (Extr. 3) tended to reduce the survival of normal skin fibroblast more than that of the ethanol extract of stem (Extr. 1), both of them showed strong results of dose-dependent patterns in the inhibition of cell death induced by NK and TA venoms. Furthermore, boiled water (Extr. 5) and 40% ethanol (Extr. 4) extracts of leaves slightly inhibited cytotoxic effect of both snake venoms.

2.2. Analysis of extracts and venom (NK or TA) proteins binding

The SDS-PAGE electrophoretograms demonstrated the venom proteins remaining in supernatant after incubation with the extracts. Some of both the venom proteins disappeared or faded when incubated with Extr. 1–3. This indicated that the precipitation of the venom proteins after incubation with these three extracts occurred. To examine the solvent effect, the mixtures of venom and solvent alone (ethanol or 40% ethanol) were tested. The results showed a gradual disappearance of some of both venom proteins, but no significant effect on the results.

2.3. Inhibitory activity of extracts against enzymatic activities of NK and TA venoms

Enzymatic and inhibition studies revealed that the ethanol extract of stems and leaves (Extr. 1 and 3) showed good inhibition of PLA$_2$ and protease enzymes present in those venoms which rendered as active extracts. These extracts were able to inhibit the toxic enzymes of both venoms at 10 mg/mL concentration. Because of interference of the extract itself, the inhibition of Extr. 4 and 5 against protease enzyme of NK venom was unreadable. NK protease had lower activity than that of TA. Even after increasing the concentration of NK venom to 20 mg/mL, the results of Extr. 4 and 5 were still unreadable. However, these two extracts tended to be weak inhibitors for venom enzymes.

2.4. Cytotoxicity of ethanol whole plant extract

The cytotoxicity assay conforms to the published standard methods (BS-EN30993-5 and ISO10993-5) (Plumb et al. 1989) using Vero, Caco2, HepG2 and HEp-2 cell lines. The results showed the ethanol whole-plant extract of PB exhibited no cytotoxicity against these four cell lines (IC$_{50}$ > 500 μg/mL).
2.5. Antioxidant of PB extracts

All extracts were subjected to examination for potential free-radical scavenging on DPPH. The result indicated that the ethanol extract of leaves (Extr. 3) exhibited weak antioxidant activity at IC$_{50}$ value of 1.61 mg/mL. Other extracts were regarded as inactive (IC$_{50} > 2.0$ mg/mL), while the standard reference (ascorbic acid) showed the IC$_{50}$ value of 0.02 mg/mL.

2.6. In vivo acute oral toxicity determination (LD$_{50}$) of ethanol whole-plant extract

The ethanol extract of a whole plant of PB was selected to test for its acute oral toxicity in Wistar rats. The result showed that all treated rats did not exhibit abnormal toxic signs or deaths. No gross pathological lesions were observed on necropsy of the control and all treated rats.

3. Conclusions

To our knowledge, this is the first report on the in vitro anti-snake venom of PB extracts against NK and TA venoms. From the reports, the cytotoxic and enzymatic analysis reveals that the ethanol extracts of stems and leaves of PB showed good anti-snake venom activity against NK and TA venoms. Binding with venom proteins may involve the inhibitory action of the extracts. The antioxidant result suggested that only the ethanol leaves extract exhibited weak DPPH radical-scavenging activity. The ethanol whole-plant extract also showed no cytotoxicity against four cell lines. Moreover, the ethanol extract of a whole plant did not exhibit abnormal toxic signs or death in in vivo acute oral toxicity results. Thus, we can suggest that the ethanol extracts of PB have a potential for use in anti-snake venom which should studied further for its potential in pharmacological and in vivo studies.

Supplementary material

Experimental details relating to this article are available online.

Acknowledgements

This work was supported by the Higher Education Research Promotion and National Research University Project of Thailand, Office of the Higher Education Commission. The authors would also like to thank Rajamangala University of Technology Thanyaburi for their support.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

Plumb JA, Milroy R, Kaye SB. 1989. Effects of the pH dependence of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide-formazan absorption on chemosensitivity determined by a novel tetrazolium based assay. Cancer Res. 49:4435–4440.

Singh RS, Pandey RP, Singh BK, Singh RG. 2000. Aliphatics from Peristrophe bicalyculata. Fitoterapia. 71:80–81. doi:10.1016/S0367-326X(99)00100-8.

Thuy TT, Lam TH, Thanh Huong NT, Hong Nhung LT, Ninh PT, Hoang Anh NT, Phuong Thao TT, Van Sung T. 2012. Natural phenoxazine alkaloids from Peristrophe bivalvis (L.) Merr. Biochem Syst Ecol. 44:205–207. doi:10.1016/j.bse.2012.05.009.

Trinh LN, Watson JW, Hue NN, De NN, Minh NV, Chu P, Shapit BR, Eyzaguirre PB. 2003. Agrobiodiversity conservation and development in Vietnamese home gardens. Agr Ecosyst Environ. 97:317–344. doi:10.1016/S0167-8809(02)00228-1.