Inhibitory Effect of Water Extract from *Stephania venosa* Tubers on Serotonin Receptors Expressed in *Xenopus* Oocytes

นิพนธ์ต้นฉบับ

Original Article

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วารสารไทยเภสัชศาสตร์และวิทยาการสุขภาพ 2554;6(3):209-213

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Thai Pharmaceutical and Health Science Journal 2011;6(3):209-213

บทคัดย่อ

วัตถุประสงค์: เพื่อศึกษาฤทธิ์ยับยั้งรีเซพเตอร์ serotonin (5-HT), N-methyl D-aspartate (NMDA) และ glycine ของสารสกัดน้ำจากหัวบอระเพ็ดพุงช้าง (*Stephania venosa*) โดยใช้แบบจำลองของไข่กบ *Xenopus* **วิธีการศึกษา:** ฉีด RNA ที่มีรหัสพันธุกรรมของรีเซพเตอร์ 5-HT หรือ NMDA หรือ glycine เข้าไปในไข่กบ *Xenopus* แล้ววัดการเปลี่ยนแปลงกระแสไฟฟ้าภายในไข่กบด้วยวิธี two-electrode voltage clamp **ผลการศึกษา:** พบว่าสารสกัดสามารถยับยั้งรีเซพเตอร์ 5-HT ได้อย่างชัดเจนเมื่อไข่สัมผัสกับสารสกัดก่อน ค่า TD_{50} ของสารสกัดเท่ากับ 3 และ 60 ไมโครกรัม/มิลลิลิตรเมื่อไข่สัมผัสและไม่ได้สัมผัสกับสารสกัดก่อนตามลำดับ สารสกัด (1 ไมโครกรัม/มิลลิลิตร) ทำให้กราฟความสัมพันธ์ระหว่างความเข้มข้นของสารสกัดกับการตอบสนองของรีเซพเตอร์ 5-HT เคลื่อนไปทางขวา ค่า ED_{50} ของ 5-HT เท่ากับ 0. 1 และ 1 ไมโครโมล เมื่อไม่มีและมีสารสกัดตามลำดับ ฤทธิ์ในการยับยั้งของสารสกัด (0.1 – 1,000 ไมโครกรัม/มิลลิลิตร) ต่อรีเซพเตอร์ NMDA และ glycine มีความแรงน้อยกว่าฤทธิ์ในการยับยั้งรีเซพเตอร์ 5-HT **สรุป:** จากผลการวิจัยครั้งนี้บ่งชี้ว่า สารสกัดยับยั้งรีเซพเตอร์ 5-HT โดยการจับกับรีเซพเตอร์ตรงตำแหน่งเดียวกันกับที่ 5-HT เข้าไปจับ และฤทธิ์ในการยับยั้งมีความจำเพาะต่อรีเซพเตอร์ 5-HT มากกว่ารีเซพเตอร์ NMDA และ glycine มีการวิเคราะห์เกี่ยวกับชนิดของสารออกฤทธิ์ที่เป็นไปได้ ที่ทำให้สารสกัดแสดงฤทธิ์ยับยั้ง

คำสำคัญ: หัวบอระเพ็ดพุงช้าง, ไข่กบ *Xenopus*, รีเซพเตอร์ 5-HT

Abstract

Objectives: This study aimed to evaluate inhibitory effects of water extract from *Stephania venosa* (*S. venosa*) tubers on serotonin (5-HT), N-methyl D-aspartate (NMDA) and glycine receptors expressed in *Xenopus* oocytes. **Method:** *Xenopus* oocytes were injected with RNA of either 5-HT, NMDA or glycine receptor and transmembrane currents were recorded using the two-electrode voltage clamp technique. **Results:** The extract markedly inhibited 5-HT receptors when oocytes were pretreated with the extract. IC_{50} values of the extract were 3 and 60 µg/ml when oocytes were and were not pretreated, respectively. The extract (1 µg/ml) caused a rightward shift of the 5-HT concentration-response curve. ED_{50} values of 5-HT were 0.1 and 1 µM in the absence and presence of the extract, respectively. Inhibitory effect of the extract (0.1 – 1,000 µg/ml) on NMDA and glycine receptors was less potent than that on the 5-HT receptors. **Conclusion:** These results suggest that the extract inhibited the 5-HT receptor by interacting with 5-HT binding site in a competitive fashion. Moreover, the inhibition of the extract was more specific to 5-HT receptor than NMDA and glycine receptors. Possible active compound (s) underlying the inhibitory effect of the extract are discussed.

Key words: *Stephania venosa* tubers, *Xenopus* oocytes, 5-HT receptor

Introduction

Stephania venosa Spreng (*S. venosa*), commonly known in Thai as Boraphet-phungchang, Sabu-le-ad or Kling klaang dong, is a plant in the family of Menispermaceae. Many parts of this plant had been used as a folk remedy for multiple purposes such as stem for anthelminthic, rhizome for neuronal function, leaf for wound healing, and tuber for food appetite and health. Many studies have reported that *S. venosa* tuber in Thailand revealed a variety of pharmacological activities including antimalarials[1] acetylcholinesterase inhibitors[2] antiproliferative activity on cancer cells[3,4] and antiinflammatory activity.[5] Over thirty isoquinoline alkaloids in the tuber have been identified[6-8] and demonstrated many biological activities, such as aromoline possessed antiplasmodial, antiamoebic and cytotoxic activities; berbamine and tetradrine could inhibit production of interleukin-1 and tumour necrosis factor; berbamunine and cepharanthine displayed antiinflammatory and immunosuppressive activities; dicentrine showed antihypertensive activity; and palmatine had sedative effect.[9,10]

Although most people have used the tuber of *S. venosa* in the form of crude drugs, boiled solution, liquor soaking or pills, the effectiveness of these formulations have not been scientifically verified. Our previous observation found that animals orally taking water extract from the tuber of *S. venosa* become sedated. Sedative activity of any compounds may involve inhibition of excitatory neurotransmitter. Serotonin (5-HT) acts as an excitatory neurotransmitter when it binds to 5-HT_2 receptor subtype. Many studies suggest that the expressed 5-HT receptor in *Xenopus* oocyte after injection of rat brain mRNA is 5-HT_2-

like receptor. Therefore, this study aimed to evaluate the effects of water extract from *S. venosa* tubers on 5-HT receptor using the model of *Xenopus* oocyte.

Materials and Method

Plant material and extract preparation

Authentication of the tuber of *S. venosa (Bl.) Spreng* was achieved by comparison with herbarium specimens at the Bangkok Forest Herbarium, Royal Forest Department, Ministry of Agriculture and Cooperatives, Bangkok, Thailand. *S. venosa* tubers were collected from Rachaburi Province, Thailand. The fresh tubers were harvested, chopped into small pieces and dried under the sun light for 2 - 3 days. The dried *S. venosa* tubers were boiled for 5 h. The solutions were then filtered and lyophilized using a freeze dryer to yield a brown powder.

Oocyte injection

Defolliculated stage V–VI oocytes were prepared from *Xenopus laevis* (Xenopus Express, Cape, South Africa) as described previously[11]. Briefly, *Xenopus* laevis were anesthetized in ice-water, and a lobe of the ovary was dissected and placed in sterile modified Barth's solution (MBS: 88 mM NaCl, 1 mM KCl, 0.41 mM $CaCl_2$, 0.33 mM $Ca(NO_3)_2$, 0.82 mM $MgSO_4$, 2.4 mM $NaHCO_3$, 7.5 mM Tris (hydroxymethyl) aminomethane, pH 7.6). Oocytes were then isolated manually and defolliculated by incubation in 2 mg/ml collagenase (type IA; Sigma, St. Louis, MO, USA) at 19 °C for 1 h in calcium-free MBS solution. To examine 5-HT or glycine receptor function, total mRNA was prepared from whole brain or spinal cord of adult male rats, respectively, using the Trizol reagent (Gibco-BRL)[12], and oocytes were injected with 46 nl of the total mRNA (5 mg/ml). To examine NMDA receptor function, cRNA was prepared from cDNA clones of NR1a and NR2B kindly provided by Dr. K. Igarashi (Faculty of Pharmaceutical Sciences, Chiba University) and oocytes were injected with 27.6 nl of NR1a/NR2B RNA mixture[13]. After injection, oocytes were incubated in MBS containing 2.5 units /ml penicillin and 2.5 μg/ml streptomycin at 18 °C.

Electrophysiological recording

Responses to 5-HT were recorded using a two-electrode voltage-clamp amplifier (GeneClamp 500B; Axon Instrument, Foster City, CA, USA) at a holding potential of -70 mV unless noted otherwise. Electrodes were filled with 3 M KCl and had resistances of 0.5 – 5 MΩ. Oocytes were positioned in a 50-μl chamber and continuously perfused with MBS solution at 1 ml/min at room temperature. The drugs were applied until a plateau or peak of the response was observed. Data were recorded and digitized for analysis (MacLab 200; ADInstruments, Castle Hill, NSW, Australia). The washout period for recovery was 3 – 5 min, depending on the concentration of drugs applied. Most data was expressed as the mean ± S.E.M. For statistical analysis, the SigmaStat (ver 3.5) program was used. Data were subject to the paired *t*-test when effects were compared between before and after drug application in the same oocytes or the unpaired *t*-test when the experiments were performed in different oocytes. If the data were compared with 100% control, Mann-Whitney Rank Sum test was used. Differences with $P < 0.05$ were considered significant.

Results

Dose-dependent inhibition of S. venosa extract on 5-HT receptors

To examine inhibitory effect of the extract on the expressed 5-HT receptors, 0.1 μM 5-HT was applied to oocytes in the absence and presence of the extract. Simultaneous application of the extract at 10, 100 and 1000 μg/ml and 5-HT to oocytes produced 9 ± 7.7%, 65 ± 5.5% and 80 ± 2.3% inhibition, respectively (Fig. 1A, 1C). Pretreatment of oocytes with the extract for 1 min before simultaneous application, the inhibition were 25 ± 3.4%, 75 ± 7.7% and 100 ± 0% for the extract at 1, 10 and 100 μg/ml, respectively (Fig. 1B, 1C). The magnitude of inhibition was significantly increased when oocytes were pre-treated with the extract (Fig. 1C). The 5-HT responses that were reduced by the extract at high concentrations hardly recovered after washout, in contrast to those reduced by lower concentrations (Fig. 1A, 1B).

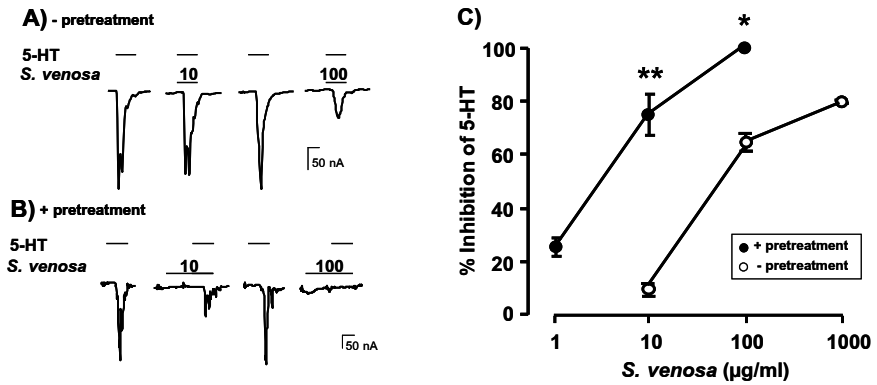

Figure 1 Dose-dependent inhibition of *S. venosa* extract on 5-HT receptors. Oocytes injected with total rat brain RNAs were applied with 0.1 µM 5-HT in the absence (control) or presence of the extract at various concentrations (1 – 1,000 µg/ml). Examples of traces demonstrated effects of the extract at 10 and 100 µg/ml when an oocyte was not (A) or was (B) pretreated with the extract. C) Data are expressed as the mean ± S.E.M. of percentages of control response from 3 - 6 oocytes. **P*< 0.05 and ***P*< 0.001, compared with that without pretreatment.

Effect of the extract on the 5-HT concentration-response curve

To study the inhibitory mechanism of the extract on the 5-HT receptors, oocytes were treated with different concentrations of 5-HT in the absence and presence of the extract 1 µg/ml. The extract caused a shift of the 5-HT response curve to the right without a lower maximal response. The ED_{50} of 5-HT was changed from 0.1 to 1 µM (Fig. 2).

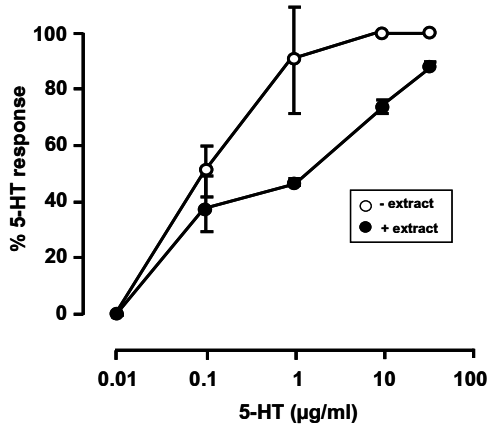

Figure 2 Effect of *S. venosa* extract on the 5-HT concentration-response curve. Oocytes injected with total rat brain RNAs were applied with various concentrations of 5-HT (0.01 – 30 µM) in the absence (control) or presence of the extract 1 µg/ml. Data are expressed as the mean ± S.E.M. of percentages of control response from 2 - 6 oocytes.

Inhibitory effect of S. venosa extract on NMDA and glycine receptors

To examine the specificity of the inhibitory effect of the extract on 5-HT receptor, the effects of the extract on NMDA and glycine receptor functions in oocytes injected with cRNA of NR1a/NR2B cDNA or rat spinal cord RNA, respectively, were tested. The current response elicited by NMDA agonists (10 µM glutamate plus 10 µM glycine) was dose-dependently reduced by the extract with IC_{50} of 200 µg/ml (Fig. 3A).

The inhibitory effect of the extract easily disappeared after washout when compared to that of the noncompetitive NMDA receptor antagonist MK-801. The current response, of glycine receptor, elicited by 300 µM glycine was dose-dependently reduced by the extract with IC_{50} of 500 µg/ml (Fig. 3B). Pretreatment of oocytes with the extract prior to application of either NMDA or glycine did not enhance the inhibitory effect of the extract (data not shown).

Discussions and Conclusion

The present study has demonstrated 5-HT tubers dose-dependently inhibited 5-HT-receptor. When the inhibition on dose-response curve of 5-HT was observed, the results suggested that the inhibitory effect is similar to that of a competitive antagonist. Moreover, the inhibitory effect of the extract on 5-HT receptor is rather specific compared to that on NMDA and glycine receptors.

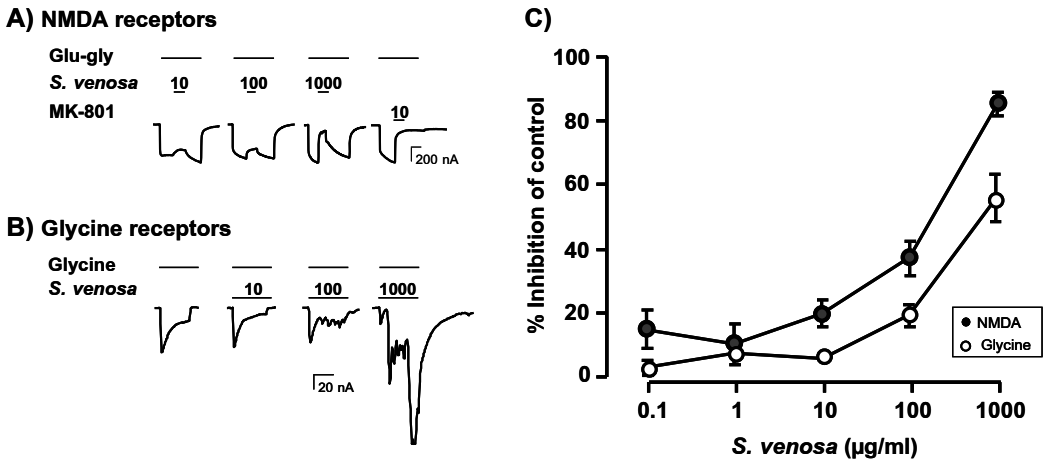

Figure 3 Inhibitory effect of *S. venosa* extract on NMDA and glycine receptors. Oocytes injected with NR1a and NR2B cRNAs or rat spinal cord RNAs were applied with 10 µM glutamate plus 10 µM glycine (Glu-gly) or 300 µM glycine, respectively, in the absence (control) or presence of the extract at 0.1 – 1,000 µg/ml. A) Examples of NMDA currents inhibited by the extract at different concentrations (10 – 1,000 µg/ml). MK-801 (10 µM), a noncompetitive NMDA receptor antagonist, was tested as a positive control. B) Examples of glycine currents inhibited by the extract at different concentrations (1 – 1,000 µg/ml). C) Data are expressed as the mean ± S.E.M. of percentages of control response from 2 - 8 oocytes.

The water extract from *S. venosa* tubers is a crude extract. Many constituents in the extract might involve in the observed inhibitory effect on 5-HT receptor. Using gas chromatography (GC) and mass spectrometer (MS) suggested that domestine and tetrahydropalmatine (THP) are the main components of the water extract from *S. venosa* tubers.[14] Biological activity of domestine was not clearly identified. In contrast, many evidences demonstrated that THP possesses various pharmacological activities such as analgesic, sedative, hypnotic, and antihypertensive.[15,16] Animal experiments have shown that the sedative effect of THP results from blocking dopaminergic neurons in the brain, and the benzodiazepine mediates, at least in part, such the effect.[17] Many reports showed that blocking serotonin activity involved the antihypertensive and antinociceptive actions of THP.[18] Although our previous observation found that animals injected with water extract from *s. venosa* tubers showed sedative effect, there is no evidence to support the involvement of THP and 5-HT blocking in sedative action. Other constituent (s) rather than THP might involve 5-HT blocking in sedative action of the extract. It is interesting to further examine which compound (s) in the extract acts as the 5-HT receptor antagonist.

Acknowledgements

This work was supported in part by the research grant from Graduate School, Srinakharinwirot University.

References

1. Likhitwitayawuid K, Dej-adisai S, Jongbunprasert V and Krungkrai J. Antimalarial from *Stephania venosa*, *Prismatomeris sessiliflora*, *Diospyros montana* and *Murraya siamensis*. *Planta Med*1999;*65:*754-756.
2. Ingkaninan K, Yuyaem T, Thongnoi W. Anti-cholinesterase activity of *Stephania. Naresuan Univ J* 2001*;9:*35-40.
3. Moongkarndi P, Kosem N, Luanratana O, Jongsomboonkusol S, Pongpan N. Antiproliferative activity of Thai medicinal plant extracts on human breast adenocarcinoma cell line. *Fitoterapia* 2004;75:375–377.
4. Sueblinvong T, Plumchai T, Leewanich P, Limpanasitthikul W. Cytotoxic effects of water extract from *Stephania venosa* tubers. *Thai Pharm Health Sci J* 2007*;* 2:203-208.
5. Potduang B, Kajsongkram T, Limsiriwong P, Giwanon R, Thisayakorn K, Meeploy M, Benmart, Y. Chief constituents and biological activities determination of *Stephania venosa*. *Acta Hort* 2005;(ISHS) 677:57-64.
6. Banerj J, Chatterjee A, Patra A, Bos P, Das R., Das B, Shamma M, Tantiseurie B. Kamaline, an unusual aporphine alkaloid from *Stephania venosa*. *Phytochemistry* 1994;*36*:1053-1056.
7. Tantisewie B, Amurrio S, Guinaudeau H, Shamma M. New bisben-zylisoquinolines from *Stephania pierri. Nat Prod* 1989;52:846-851.

8. Charles B, Bruneton J, Pharadai K, Tantisewie B, Guinaudeau H, Shamma M. Some unusual proaporphine and aporphine alkaloids from *Stephania venos.a. J Nat Prod* 1987;50:1113-1117.
9. Likhitwitayawuid K, Angerhofer CK, Chai H, Pezzuto JM, Cordell GA, Ruangrungsi N. Cytotoxic and antimalarial alkaloids from the tubers of *Stephania pierrei*. *J Nat Prod* 1993*;56:*1468-1478.
10. Kewpradub N, Itharat A, Tantikamkul A, Rugleng S, Inruspong P. Cytotoxic alkaloids from the tuber of *Stephania venosa*. S*ongklanakarin J Sci Technol* 2001*;23:*225-234.
11. Leewanich P, Tohda M, Matsumoto K, Takayama H, Sophasan S, Watanabe H. Corymine potentiates NMDA-induced currents in NR1a/NR2B cRNA injected *Xenopus* oocytes. J Pharmacol Sci 2005*;* 98:58-65.
12. Leewanich P, Tohda M, Matsumoto K, Subhadhirasakul S, Takayama H, Aimi N, Watanabe H. Inhibitory effects of corymine-related compounds, indole alkaloids, on glycine receptors expressed in *Xenopus* oocytes. *Jpn J Pharmacol* 1998;77:169-172.
13. Leewanich P, Tohda M, Matsumoto K, Takayama H, Sophasan S, Watanabe H. Corymine potentiates NMDA-induced currents in NR1a/NR2B cRNA injected *Xenopus* oocytes. *J Pharmacol Sci* 2005; 98:58-65.
14. Leewanich P, Prachayasitikul S*. In vitro* study of *Stephania venosa* extracts on anticancer activity. Proceedings of the Srinakharinwirot Academic Meeting. 2008 January 31 – February 1. Bangkok, Thailand. Bangkok. Sahamitre Printing and Publishing, 2008: pp. 373-381.
15. Dhamanada S. The story of JIN BU HUAN. *Herbal Gram* 1994;31: 28–31.
16. Lin MT, Chueh FY, Hsieh MT, Chen CF. Antihypertensive effects of DL-tetrahydropalmatine: an active principle isolated from corydalis. *Clin Exp Pharmacol Physiol* 1996;23:738–745.
17. Leung WC, Zheng H, Huen M, Law SL, Xue H. Anxiolytic-like action of orally administered dl-tetrahydropalmatine in elevated plus-maze. *Prog Neuropsychopharmacol Biol Psychiatry* 2003;27:775–779.
18. Chu H, Jin G, Friedman E, Zhen X. Recent development in studies of tetrahydroprotoberberines: mechanism in antinociception and drug aaddiction. *Cell Mol Neurobiol* 2008;28:491-499.

Editorial note
Manuscript received in original form on September 29, 2011;
accepted in final form on December 30, 2011