

## ฤทธิ์เพิ่มการแบ่งตัวของลิมโฟไซต์จากสารสกัดเถาวัลย์เปรียงสองชนิด

Enhancing Activities of Two *Derris scandens* Extracts on Normal Lymphocyte Proliferationวีณา ศรีแสงศรี<sup>1</sup>วไลลักษณ์ วรรณวิเศษ<sup>1</sup>ปราณี ขวลิตรำรง<sup>2</sup>บุษราวรรณ ศรีวรรณ<sup>1</sup>Weena Threesangsri<sup>1</sup>Walailuk Wanavichet<sup>1</sup>Pranee Chavalittumrong<sup>2</sup>Busarawan Sriwanthana<sup>1</sup><sup>1</sup>สถาบันวิจัยวิทยาศาสตร์สาธารณสุข<sup>2</sup>สถาบันวิจัยสมุนไพร

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Herbs are widely consumed in every part of the world nowadays. According to traditional medicines, crude extracts of several herbs are known for strengthening and promoting health. In Thailand, *Derris scandens* Benth (family: Leguminosae) is one of medicinal plants commonly used for health promotion (Pongboonrod, 1976; Tiangburanatham, 1996). Pharmacological study revealed that *D. scandens* hydroalcoholic extract possesses *in vitro* stimulation on lymphocyte proliferation of normal peripheral blood mononuclear cells (PBMC) and enhances natural killer (NK) cells activities in normals and HIV-1 infected patients (Sriwanthana, 2001). Thai people, however, usually take decoction of *D. scandens* dried and ground stems as beverages (Pongboonrod, 1976). There is no evidence demonstrating that water extract of *D. scandens*

also has an immunostimulating activity on the immune system. We, therefore, examined enhancing activity of two *D. scandens* extracts, i.e., the water and the hydroalcoholic extracts, on lymphocyte proliferation of PBMC from normal healthy donors.

To prepare *D. scandens* extracts, the stems of *D. scandens* were collected around Prachinburi, Thailand and were identified by comparison with a voucher specimen No. BKF 126882 at the Forest Herbarium, Royal Forest Department, Bangkok, Thailand. For the hydroalcoholic extract, 4 grams of dried and ground stems were extracted with 30 millilitres of 50% ethanol using a reflux method for 2 hours. Filtrate was collected and residues were further extracted with 20 millilitres of 50% ethanol for further 2 hours. The filtrates collected from both rounds of extraction were pooled

and were dried under vacuum in a rotary evaporator. The amount of the dried extract obtained was 739 milligrams. For the water extract, 4 grams of dried and ground stems were extracted with distilled water using the same procedure as the hydroalcoholic extract. The dried amount of 1.08 grams was obtained after extraction with distilled water. Both dried extracts were dissolved in sterile distilled water to make stock concentrations of 10 mg/ml and filtered before testing.

To investigate the effects of the water and the hydroalcoholic extracts on lymphocyte proliferation, PBMC were separated from heparinized blood of 35 normal healthy donors using Ficoll-Hypaque density gradient (Boyum, 1966). The PBMC ( $2 \times 10^6$  cells/ml) were cultured in triplicate in 96-well microtiter plates (Costar, Cambridge, MA, USA) with each *D. scandens* extract at the concentrations of 2 ng/ml, 20 ng/ml, 200 ng/ml, 2  $\mu$ g/ml, 10  $\mu$ g/ml, 20  $\mu$ g/ml and 200  $\mu$ g/ml to give final concentrations of 1 ng/ml, 10 ng/ml, 100 ng/ml, 1  $\mu$ g/ml, 5  $\mu$ g/ml, 10  $\mu$ g/ml and 100  $\mu$ g/ml, respectively, in complete RPMI 1640 (RPMI 1640 medium supplemented with 2mM glutamine, 10 mM HEPES, 100 U/ml penicillin G and 100  $\mu$ g/ml Streptomycin) containing 10% fetal bovine serum. The cultures were incubated at 37°C with 5% CO<sub>2</sub> for 72 hours. Eighteen hours before harvest, 20  $\mu$ l of 0.5  $\mu$ Ci <sup>3</sup>H-thymidine (specific activity 8.3 mCi/mg: Amersham, Buckinghamshire, UK) was added. <sup>3</sup>H-thymidine incorporation was determined by harvesting with a multichannel automatic cell harvester (FilterMate Cell Harvester, Packard Instrumen-

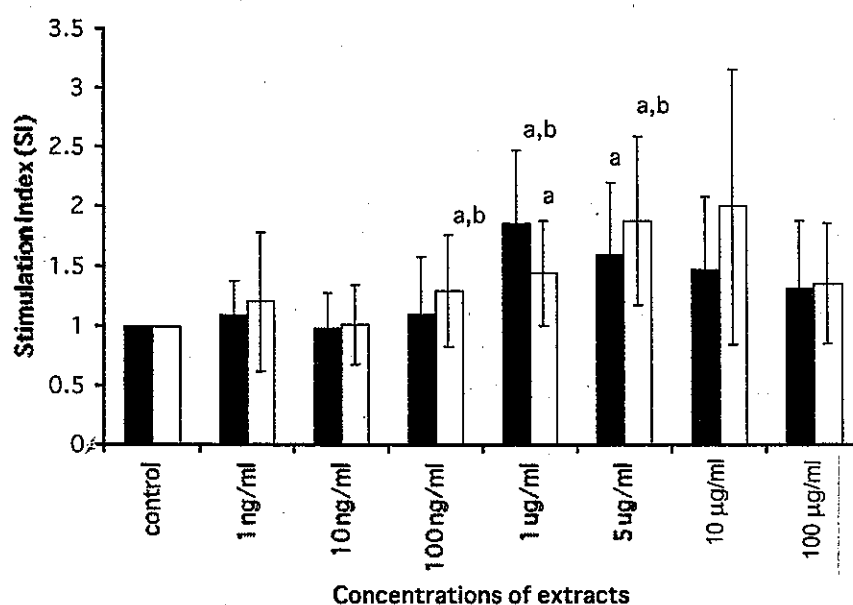
tal Co., CT, USA) onto glass fiber filters (UniFilter-96, GF/C, Packard Instrumental Co., CT, USA). The radioactivity was measured by a liquid scintillation counter (Topcount Microplate Scintillation & Luminescence Counter, Packard Instrumental Co., CT, USA). The degree of activation was expressed as a stimulation index [S.I., i.e., the ratio of the <sup>3</sup>H-thymidine uptake in count per minute (CPM) of samples with extract to those without extract]. Phytohemagglutinin HA16/17 (Murex Diagnostics Limited, Dartford, England) at 2  $\mu$ g/ml was also added to the culture system to check for cell survival.

No significant differences of the lymphocyte proliferative responses, reported as stimulation index (S.I.), were shown at the concentrations of 1 ng/ml and 10 ng/ml. Significant increases in lymphocyte proliferation were found at the concentrations ranging from 100 ng/ml to 100  $\mu$ g/ml. For the hydroalcoholic extract, the lymphocyte proliferation was significantly increased at the concentrations of 1  $\mu$ g/ml, 5  $\mu$ g/ml, 10  $\mu$ g/ml and 100  $\mu$ g/ml. Significant changes of lymphocyte proliferation were not demonstrated at the concentrations of 1 ng/ml, 10 ng/ml and 100 ng/ml (Figure 1)

We also compared enhancing activities of both extracts at the same concentrations used to stimulate PBMC from the same donors. It was shown that the responses to the water extract were significantly higher at the concentrations of 100 ng/ml, 5  $\mu$ g/ml and 10  $\mu$ g/ml as compared with their corresponding hydroalcoholic extracts. Lymphocyte prolifera-

tion at the concentration of 1  $\mu\text{g}/\text{ml}$  was significantly higher in PBMC cultures with the

hydroalcoholic extract as compared with the water extract cultures (Figure 1).



**Figure 1** Lymphocyte proliferative responses to the hydroalcoholic and the water extracts of *D.scandens*. PBMC, isolated from 35 normal donors, were cultured in the presence of the hydroalcoholic extract (filled bars) and the water extract (open bars). Proliferative responses, measured by incorporation of  $^3\text{H}$ -thymidine, were presented as stimulation index (mean + SE)

a: significant difference ( $p$ -value < 0.05) of each extract as compared to its control, b: significant difference ( $p$ -value < 0.05) between the hydroalcoholic and the water extracts at each concentration

Our studies suggested that the water and the hydroalcoholic extracts of *D. scandens* are capable to stimulate lymphocyte proliferation of normals. Significant difference in the degrees of responses between both extracts might be due to different compositions and/or various concentrations of the same substances resulting from extraction methods. Further analysis of *D. scandens* constituents with potentials to stimulate immunocompetent cells to proliferate should be investigated.

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