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Journal of Ethnopharmacology 76 (2001) 125–129

Journal of ETHNO-PHARMACOLOGY

www.elsevier.com/locate/jethpharm

Short communication

In vitro effect of *Derris scandens* on normal lymphocyte proliferation and its activities on natural killer cells in normals and HIV-1 infected patients

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Received 29 July 2000; received in revised form 28 February 2001; accepted 28 February 2001

Abstract

We investigated the effect of *Derris scandens* hydroalcoholic extract on lymphocyte proliferation, natural killer (NK) cell activity and secretion of IL-2 and IL-4. Lymphocyte proliferative response of normal individuals was significantly increased at concentrations of 10 ng/ml, 100 ng/ml, 1 μg/ml and 5 μg/ml, whereas the response was significantly decreased at 100 μg/ml. *D. scandens* at the concentrations of 10 ng/ml, 100 ng/ml, 1 μg/ml and 10 μg/ml significantly enhanced the function of NK cells of normal individuals. The NK cell activity of HIV-infected individuals was significantly increased at a concentration of 10 μg/ml. Furthermore, the extract was shown to induce the IL-2 secretion from normal peripheral blood mononuclear cells (PBMC), whereas the IL-4 was not induced in the presence of the *D. scandens* extract. Our data suggested that the hydroalcoholic extract of *D. scandens* possesses in vitro immunostimulating activity on human immunocompetent and immunocompromised PBMC.

Keywords: *Derris scandens*; Immunostimulant; NK activity; HIV

1. Introduction

Derris scandens Benth. (family: Leguminosae) is a woody vine growing throughout Southeast Asia, including Thailand (Muanwongyathi and Supatwanich, 1981; Tiangburanatham, 1996). *D. scandens* is one of the medicinal plants commonly used in Thailand (Pongboonrod, 1976). In Thai traditional medicine, the stem of *D. scandens* has been widely used as an expectorant, antitussive, diuretic, antidysentery, and for treatment of cachexia (Tiangburanatham, 1996). In addition, Thai people usually take an extract containing an infusion of roasted stems of *D. scandens* or its alcoholic macerate as a tonic (Pongboonrod, 1976). Its chemical constituents that have been reported are derrisoflavones A–F (Sekine et al., 1999), eturunagarone, warangalone, 8-γ,γ-dimethylallyl-wighteone, 3′-γ,γ-dimethylallyl-wighteone, scandinone, robustic acid, and 4,4′-di-*O*-methyl scandenin (Rao et al., 1994; Wang et al., 1997), scandenin, nallanin, chadanin (Clarke, 1943; Subba Rao and Seshadri, 1946; Johnson et al., 1966), osajin, scandenone, scandinone (Pelter and Stainton, 1966), chandalone, lonchocarpic acid and lonchocarpenin (Johnson et al., 1966; Falshaw et al., 1969).

D. scandens is one of the components in traditional medicine for health promotion (Pongboonrod, 1976); its effect may be due to immunostimulating activity on the immune system. We, therefore, investigated immunopharmacological effects of *D. scandens* on lymphocyte proliferation, natural killer (NK) cell activity and cytokine secretion of peripheral blood mononuclear cells (PBMC) from either normal donors or HIV-infected individuals.

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0378-8741/01/$ - see front matter
PII: S0378-8741(01)00223-9

2. Materials and methods

2.1. Preparation of D. scandens extract

D. scandens stems were collected around Prachinburi, Thailand, and were authenticated at the Forest Herbarium, Royal Forest Department, Bangkok, Thailand, where a voucher specimen was deposited (BKF 126882). Sixty grams of dried and ground stems were extracted with 200 ml of 50% ethanol using a reflux method for 2 h. The supernatant was dried under vacuum in a rotary evaporator. The amount of the dried extract obtained was 11.25 g. The dried extract was dissolved in sterile distilled water to make a stock concentration of 10 mg/ml, filtered and kept in a refrigerator until use.

2.2. Subjects

Sixty-eight healthy Thai donors of the national blood bank, The Thai Red Cross Society, were recruited in this study. Their ages ranged from 20 to 50 years old. None had a history of hepatitis B infection, nor had a risk for HIV-1 exposure.

Twenty-one HIV-infected individuals were those who attended the anonymous clinic, The Thai Red Cross Society, for their regular visit. They were selected as convenient samples for the study on NK cell activity.

2.3. Preparation of mononuclear cells

Heparinized peripheral blood was obtained aseptically from those subjects. Mononuclear cells were separated from the heparinized blood using Ficoll–Hypaque density gradient (Boyum, 1966). Two parts of heparinized blood were gently layered on three parts of Ficoll–Hypaque mixture (IsoPrep, Robbins Scientific Corporation, Sunnyvale, CA, USA) and spun at $400 \times g$ for 30 min at 25°C. The mononuclear cells were collected from the white band at the interface of the gradient and plasma, and washed three times with RPMI 1640 (Grand Island Biological Company, Grand Island, NY, USA) by centrifugation at $400 \times g$ for 10 min at 4°C. The mononuclear cells were counted and adjusted to an appropriate concentration in complete RPMI 1640 (RPMI 1640 medium supplemented with 2 mM glutamine, 10 mM HEPES, 100 U/ml penicillin G, and 100 μg/ml streptomycin) containing 10% fetal bovine serum (FBS; Grand Island Biological Company, Grand Island, NY, USA) for further assays.

2.4. Lymphoproliferation assay

Purified mononuclear cells (2×10^6 cells/ml) were cultured in triplicate in 96-well microtiter plates (Costar, Cambridge, MA, USA) with the *D. scandens* extract at concentrations of 20 ng/ml to 200 μg/ml to give final concentrations of 10 ng/ml, 100 ng/ml, 1 μg/ml, 5 μg/ml, 10 μg/ml and 100 μg/ml, respectively, in complete RPMI 1640 containing 10% FBS. The cultures were incubated at 37°C with 5% CO_2 for 72 h. Then, 18 h before harvest, 20 μl of 0.5 μCi ^{3}H-thymidine (specific activity 8.3 mCi/mg: Amersham, Buckinghamshire, UK) was added. ^{3}H-thymidine incorporation was determined by harvesting with a multichannel automatic cell harvester (FilterMate Cell Harvester, Packard Instrumental 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 ^{3}H-thymidine uptake in counts per minute (CPM) of samples with extract to those without extract. Phytohemagglutinin HA16/17 (Murex Diagnostics Limited, Dartford, UK) at 2 μg/ml was also added to the culture system to check for cell survival.

2.5. NK cell activity assay

PBMC were washed, resuspended and adjusted to 2×10^6 cells/ml in complete RPMI 1640 containing 10% FBS. PBMC were incubated in the presence or the absence of the *D. scandens* extract at final concentrations of 10 ng/ml, 100 ng/ml, 1 μg/ml, 10 μg/ml, and 100 μg/ml at 37°C for 18 h. After incubation, the cultures were washed and then used as effector cells for the assay of NK cell activity.

K 562 cells were used as target cells and were grown in complete RPMI 1640 containing 10% FBS. The target cells (2×10^6 cells) were labeled with 100 μCi of $Na_2^{51}CrO_4$ (specific activity 37.0 MBq/μg: Amersham, Buckinghamshire, UK) at 37°C 5% CO_2 for 60 min, and washed three times with cold RPMI 1640 containing 10% FBS.

The cytotoxicity assay was performed in 96-well round-bottom microtiter plates (Corning Incorporated, Corning, NY, USA) using 2×10^3 target cells/well and PBMC effector-to-target cell ratios (E:T) of 90:1, 30:1, 10:1, and 3:1. Each condition was set up in triplicate. The plates were centrifuged at $200 \times g$ for 5 min and were then incubated for 4 h at 37°C with 5% CO_2. After incubation, supernatants from each well (100 μl) were transferred into tubes and counted in a Gamma counter (Cobra Series Gamma Counter Systems, Packard Instrumental Co., CT, USA). The percentage of cytolysis was calculated according to the following formula:

$$\% \text{ cytolysis} = \frac{\text{experimental release} - \text{spontaneous release}}{\text{maximal release} - \text{spontaneous release}} \times 100$$

Spontaneous release was measured by incubation of target cells with medium alone, and maximal release was measured by lysis of target cells with 5% Triton X-100. NK cell activity was expressed as lytic units (LU)/10^7 PBMC as determined by least-squares analysis derived from the percentage of specific lysis of all E:T ratios. One LU was defined as the number of effector cells required for 20% specific lysis of 1×10^4 target cells.

2.6. Measurement of IL-2 and IL-4

PBMC (2×10^6 cells) were cultured in the presence or the absence of the extract at final concentrations of 10 ng/ml, 100 ng/ml, 10 μg/ml and 100 μg/ml in 24-well plates (Corning Incorporated, Corning, NY, USA). The cultures were incubated at 37°C in 5% CO_2. Supernatants were harvested from each well on days 1, 3 and 5. The supernatants collected were stored at −20°C for analysis of cytokine production. The amounts of IL-2 and IL-4 secreted were examined using PREDICTA IL-2 and PREDICTA IL-4 kits (Genzyme, Cambridge, MA, USA) with detection limits of 4 pg/ml and 6 pg/ml respectively.

2.7. Statisical analysis

Data were expressed as mean ± SE and were analyzed using a paired *t*-test for lymphocyte transformation test and for NK cell activities.

3. Results

3.1. Lymphocyte proliferation

To evaluate the immunostimulating effect of the *D. scandens* extract, we examined the extract-induced proliferative responses in PBMC from 68 normal donors. The response, reported as stimulation index, was significantly increased at concentrations of the *D. scandens* extract ranging from 10 ng/ml to 5 μg/ml ($P < 0.01$) (Table 1). No significant difference was observed at a concentration of 10 μg/ml, whereas the lymphocyte proliferation was significantly decreased at a concentration of 100 μg/ml compared with the cultures in the absence of the *D. scandens* extract. Lymphocyte proliferative response to phytohemagglutinin (PHA), a potent mitogen, was demonstrated as shown in Table 1.

3.2. IL-2 and IL-4 production

To determine if *D. scandens* could account for the induction of cytokines, supernatants from the cultured PBMC were tested for IL-2 and IL-4 secretion. Owing to the limited number of PBMC obtained, we could perform on only four donors whose PBMC were also used to assess for lymphocyte proliferation and NK cell activity. The supernatants of 2 donors could be collected on days 1, 3 and 5, while those of the other two donors could be harvested only on day 1 and day 3.

IL-2 was detected on day 3 and day 5 of incubation at concentrations of 100 ng/ml and 10 μg/ml, whereas no IL-2 was detectable from the cultures with the *D. scandens* extract at a concentration of 100 μg/ml, compared with the control cultures. The amount of IL-2 detected on day 3 at concentrations of 100 ng/ml and 10 μg/ml was 22.50 ± 2.40 pg/ml and 26.75 ± 4.15 pg/ml respectively. On day 5 of incubation, IL-2 was detected at amounts of 23.50 ± 1.50 pg/ml and 39.00 ± 3.00 pg/ml from supernatants of the cultures containing the *D. scandens* extract at concentrations of 100 ng/ml and 10 μg/ml respectively.

The levels of IL-4 were undetectable in all four cultures, compared with the controls, at all concentrations of *D. scandens* tested (data not shown).

3.3. NK cell activity

To examine the ability of *D. scandens* on NK cell activity, PBMC from 21 normal donors and HIV-infected persons were treated with various concentrations of the hydroalcoholic extract. For the normal group, a significant increase in NK cell activity was found when the concentrations of *D. scandens* were 10 ng/ml, 100 ng/ml, 1 μg/ml and 10 μg/ml ($P < 0.01$). The activity was significantly reduced at 100 μg/ml of *D. scandens* in the cultures (Table 2).

The presence of the *D. scandens* extract significantly enhanced NK cell activity of PBMC from the HIV-infected group only at a concentration of 10 μg/ml ($P < 0.05$), whereas no significant enhancement was observed at concentrations of 10 ng/ml, 100 ng/ml, 1 μg/ml and 100 μg/ml (Table 2).

Table 1
Effect of *D. scandens* on lymphocyte proliferation of normal PBMC

Extract concentrations	Stimulation Index (S.I.)[a] ($n = 68$)
None	1.00 ± 0.00
10 ng/ml	1.64 ± 0.14 [b]
100 ng/ml	1.60 ± 0.12 [b]
1 μg/ml	1.66 ± 0.16 [b]
5 μg/ml	1.52 ± 0.13 [b]
10 μg/ml	1.27 ± 0.14
100 μg/ml	0.36 ± 0.06 [b]
PHA 2 μg/ml	44.67 ± 7.44 [b]

[a] Mean ± SE.
[b] $P \leq 0.05$.

Table 2
NK cell activity by normal and HIV-infected PBMC in the presence of the *D. scandens* extract

Extract concentrations	Normal PBMC[a] ($n=21$)	HIV-infected PBMC[a] ($n=21$)
None	57.18 ± 10.50	55.41 ± 12.32
10 ng/ml	72.41 ± 10.24 [b]	63.88 ± 11.69
100 ng/ml	81.04 ± 13.46 [b]	70.50 ± 11.83
1 μg/ml	100.50 ± 17.10 [b]	62.08 ± 11.29
10 μg/ml	112.51 ± 21.48 [b]	100.13 ± 22.99 [b]
100 μg/ml	14.31 ± 2.47 [b]	83.75 ± 20.39

[a] Mean ± SE (lytic units/10^7 PBMC).
[b] P-value ≤ 0.05.

4. Discussion

Immunopharmacological agents or immunomodulators can be divided into two groups, i.e. immunostimulating and immunosuppressive agents, which are both useful for therapeutic needs (Werner and Jolles, 1996). A large number of plants used in traditional medicines have been shown to possess non-specifically stimulating activities on humoral and cell-mediated immune responses (Azuma, 1987). Patients with inadequate functioning and regulation of the immune system will, therefore, possibly get benefit from medicinal plants with 'potential' immunostimulating activities. Several parameters, such as NK cell activity, antibody-dependent cellular cytotoxicity (ADCC), induction of specific antibody and T and B cell proliferation, can be used to evaluate the immunostimulating activity of the natural products (See et al., 1997; Mungantiwar et al., 1999; Benencia et al., 2000). Our investigations were designed to study the in vitro effects of *D. scandens*, which is commonly used among Thai people, on lymphocyte proliferation, NK cell activity and cytokine (IL-2 and IL-4) production of human PBMC.

It was reported that flavonoids significantly enhanced lymphocyte proliferation and caused a significant recovery of IL-2 production in mice inhibited with hydrocortisone acetate (Liang et al., 1997). Therefore, the increase in lymphocyte proliferation, NK cell function and the release of IL-2 from normal PBMC in our study may be due to the effect of isoflavones, compounds of the flavonoid group, found in the extract of *D. scandens*. Our findings may explain that the augmentation of NK cell function could possibly result in the release of IL-2 with a consequent enhancing of lymphocyte proliferation. In addition, the extract-induced proliferative response may continuously induce the secretion of IL-2.

The reduction in all activities at 100 μg/ml was not due to the toxicity of the extract, because the viability of PBMC, as determined by tryphan blue staining, was not different from the corresponding control cultures. The cell viability of each donor was 95% or more in the presence or the absence of the extracts (data not shown). It was possible that the *D. scandens* extract at a concentration of 100 μg/ml or higher could induce secretion of cytokines and/or other soluble factors that have inhibitory effects on lymphocyte proliferation and NK cell activity.

Owing to the limited amount of blood collected from the HIV-infected group, the numbers of PBMC obtained were not enough to test on all three parameters. Infection with HIV-1 results in the development of AIDS with opportunistic infections and cancers. NK cells have a potential role on host immune responses against viral infections and cancers. We therefore decided to test the effect of *D. scandens* on only NK cell activity. Our results showed the enhancement of NK cell function from HIV-infected PBMC, suggesting a possible role of *D. scandens* on the delay of disease progression, such as resistance against infectious agents or rejection of cancer cells.

Treatment with antiretroviral drugs is a powerful tool to decrease viral loads, resulting in a slow rate of progression to disease. The antiviral drugs are, however, reported to be toxic to patients. Furthermore, people living with AIDS, especially in developing countries, cannot afford the cost of available antiretroviral regimens. The use of immunostimulants from medicinal plants that would greatly enhance the function of immune cells is an alternative choice for those patients.

IL-2 is an important cytokine that regulates proliferation and differentiation of lymphocytes. During HIV-infection, IL-2 production by PBMC is significantly reduced (Westby et al., 1998). The administration of IL-2 in individuals infected with HIV-1 is expected to improve immune responses by stimulating immunocompetent cells to proliferate (Nourse et al., 1994). It has been noted that treatment with IL-2 significantly increased the number of $CD4^+$ cells in HIV-infected individuals (Schwartz et al., 1991; Jacobson et al., 1996; Kovacs et al., 1996). Detection of IL-2 in the supernatants from the cultures of normal PBMC in the presence of appropriate concentrations of the *D. scandens* extract implied a possibility of the extract to induce IL-2 secretion from HIV-infected PBMC. Whether *D. scandens* has the potential to reconstitute immune competency of HIV-infected individuals should be investigated further. Furthermore, in vivo studies should be performed to evaluate the capability of *D. scandens* to stimulate specific and non-specific immunity in normal healthy volunteers as well as in HIV-infected individuals.

Acknowledgements

We would like to acknowledge Ms Supaporn Pittiporn of the Chaopraya Apaipubate hospital, Prachinburi, for identification and providing *D. scandens*, and

Ms Weena Treesangsri and Ms Walailuk Wanawichet for assistance with laboratories. We also are particularly grateful to Professor Praphan Phanuphak and Mr Somboon Nookhai of the Thai Red Cross AIDS Research Centre for coordination on the NK studies by HIV-infected PBMC. We would also like to thank staff in the radioisotope unit and the toxicology unit at the National Institute of Health and Medicinal Plant Research Institute, respectively, for their help.

References

Azuma, I., 1987. Development of immunostimulants in Japan. In: Azuma, I., Jalles, G. (Eds.), Immunostimulants Now and Tomorrow. Japan Scientific Societies Press, Tokyo, pp. 44–56.

Benencia, F., Courreges, M.C., Coulombie, F.C., 2000. In vivo and in vitro immunomodulatory activities of *Trichilia glabra* aqueous leaf extracts. Journal of Ethnopharmacology 69, 199–205.

Boyum, A., 1966. Separation of leukocytes from blood and bone marrow. Scandinavian Journal of Clinical and Laboratory Investigation 21 (Suppl. 97).

Clarke, E.P., 1943. Scandenin — a constituent of the roots of *Derris scandens*. Journal of Organic Chemistry 8, 489–492.

Falshaw, C.P., Harmer, R.A., Ollis, W.D., Wheeler, R.E., Lalitha, V.R., Subba Rao, N.V., 1969. Natural occurrence of 3-aryl-4-hydroxycoumarins. II. Phytochemical examination of *Derris scandens*. Journal of the Chemical Society 3, 374–382.

Jacobson, E.L., Pilaro, F., Smith, K.A., 1996. Rational interleukin 2 therapy for HIV positive individuals: daily low doses enhance immune function without toxicity. Proceeding of National Academy of Sciences of United States of America 93, 10405–10410.

Johnson, A.P., Pelter, A., Stainton, P., 1966. Extractives from *Derris scandens*. I. The structures of scandenin and lonchocarpic acid. Journal of the Chemical Society 2C, 192–203.

Kovacs, J.A., Vogel, S., Albert, J.M., Falloon, J., Davey, R.T. Jr, Walker, R.E., Polis, M.A., Spooner, K., Metcalf, J.A., Baseler, M., Fyfe, G., 1996. Controlled trial of interleukin-2 infusions in patients with the human immunodeficiency virus. New England Journal of Medicine 335, 1350–1356.

Liang, H.R., Vuorela, P., Vourela, H., Hiltunen, R., 1997. Isolation and immunomodulatory effect of flavonol glycosides from *Epimedium hunanense*. Planta Medica 63, 316–319.

Muanwongyathi, P., Supatwanich, P., 1981. Pharmacognostic study of *Derris scandens* Benth. Mahidol University Journal of Pharmaceutical Science 8, 57–64.

Mungantiwar, A.A., Nair, A.M., Shinde, U.A., Dikshit, V.J., Saraf, M.N., Thakur, V.S., Sainis, K.B., 1999. Studies on the immunomodulatory effects of *Boerhaavia diffusa* alkaoidal fraction. Journal of Ethnopharmacology 65, 125–131.

Nourse, J., Firpo, E., Flanagan, W.M., Coats, S., Polyak, K., Lee, M.H., Massague, J., Crabtree, G.R., Roberts, J.M., 1994. Interleukin-2-midiated elimination of the p27Kip1 cyclin-dependent kinase inhibitor prevented by rapamycin. Nature 372, 570–573.

Pelter, A., Stainton, P., 1966. Extractives from *Derris scandens*. II. Isolation of osajin and two new isoflavones, scandenone, scandinone. Journal of the Chemical Society 7C, 701–704.

Pongboonrod, S., 1976. Mai thed mueong Thai. Folkloric Uses of Thai and Foreign Medicinal Plants. Chaiwat Press, Bangkok.

Rao, M.N., Krupadanam, G.L.D., Srimannarayana, G., 1994. Four isoflavones and two 3-aryl coumarins from stems of *Derris scandens*. Phytochemistry 37, 267–269.

Schwartz, D.H., Skowron, G.S., Merigan, T.C., 1991. Safety and effects of interleukin-2 plus zidovudine in asymptomatic individuals infected with human immunodeficiency virus. Journal of Acquired Immune Deficiency Syndrome 4, 11–23.

See, D.M., Broumand, N., Sahl, L., Tilles, J.G., 1997. In vitro effects of echinacea and ginseng on natural killer and antibody-dependent cell cytotoxicity in healthy subjects and chronic fatigue syndrome or acquired immunodeficiency syndrome patients. Immunopharmacology 35, 229–235.

Sekine, T., Inagaki, M., Ikegami, F., Fujii, Y., Ruangrungsi, N., 1999. Six diprenylisoflavones, derrisisoflavones A–F, from *Derris scandens*. Phytochemistry 52, 87–94.

Subba Rao, N.V., Seshadri, T.R., 1946. Chemical examination of plant insecticides. II. Chemical components of *Derris scandens*. Proceeding of the Indian Academic of Sciences 24A, 365–374.

Tiangburanatham, W., 1996. Dictionary of Thai Medicinal Plants. Prachumtong Printing, Bangkok, pp. 349–350.

Wang, B.H., Ternat, B., Polya, G., 1997. Specific inhibition of cyclic AMP-dependent protein kinase by warangalone and robustic acid. Phytochemistry 44, 787–796.

Werner, G.H., Jolles, P., 1996. Immunostimulating agents: what next? A review of their present and potential medical applications. European Journal of Biochemistry 242, 1–19.

Westby, M., Marriott, J.B., Guckian, M., Cookson, S., Hay, P., Dalgleish, A.G., 1998. Abnormal intracellular IL-2 and interferon-gamma (IFN-γ) production as HIV-1-associated markers of immune dysfunction. Clinical and Experimental Immunology 111, 257–262.