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# Immunomodulating Activity of *Derris scandens* Benth.

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**ABSTRACT:** According to the Thai traditional medicine, *Derris scandens* Benth. stem is used for the relieve of muscle ache and pain, as a diuretic, expectorant and antitussive. Since *Derris scandens* is one of the most commonly used medicinal plants in Thailand and it is also used as a tonic, it is interesting to study if this plant possesses immunomodulating activity. Therefore, the effect of 50% ethanolic extract of *Derris scandens* on lymphocyte proliferation, mitogen response and natural killer (NK) activity was studied in mouse splenic lymphocytes. It was found that the extract at the concentrations of 0.78-50 µg/ml could stimulate lymphocyte proliferation and enhanced lymphocyte response to T-cell mitogens, i.e. phytohaemagglutinin (PHA) and concanavalin A (ConA). The enhancement of mitogen response may, in part, be due to the stimulatory effect of the extract on interleukin-2 release. However, the extract at the concentrations of 0.39-1.56 µg/ml did not affect NK activity of mouse splenic lymphocytes. Since the extract showed significant immunomodulating activity *in vitro*, it was then tested for *in vivo* activity in Balb/c mice. The animals were given the extract orally at the doses equivalent to crude drug 0.03 or 3 g/kg BW/day, or 1 and 100 times of therapeutic dose for 15 days. It was found that lymphoproliferation or T-cell mitogen response of splenic lymphocytes from the two extract-treated groups was not significantly different from their control groups. In conclusion, 50% ethanolic extract of *D. scandens* showed marked *in vitro* immunomodulating activity in mouse splenic lymphocytes; however, when given *in vivo*, the extract neither increased lymphocyte proliferation nor enhanced T-cell mitogen response. The reasons for this discrepancy were discussed.

**Key words:** *Derris scandens*, immunomodulation, lymphocyte, interleukin-2.

## TRODUCTION

*Derris scandens* Benth. is a medicinal plant in the ıily Leguminosae commonly known in Thai as ıao-Wan-Priang" (1). It is a woody vine widely ributed throughout Thailand. Its dried stem is used Thai traditional medicine as expectorant, antitussive, -etic, antidysentery, and for the treatment of muscle ache pain, while the root is used as fish poison (1-4). -tochemical study shows that the stem contains -unagarone, warangaione, 8-γ,γ-dimethylallylwighte-, 3'-γ,γ-dimethylallylwighteone, scandinone, robustic l, and 4,4''-di-O-methyl scandenin (5). The root is ɔrted to contain scandenin, lonchocarpic acid (6-7), anin, chadanin (8), osajin, scandenone (warangalone), scandinone (9), chandalone, and lonchocarpenin (10). It was recently found that warangalone, robustic acid, 8-γ,γ-dimethylallylwighteone, 3'-γ,γ-dimethylallylwighteone, and nallanin are selective and potent inhibitors of rat liver cyclic AMP-dependent protein kinase catalytic subunit (cAK) with $IC_{50}$s at 3.5, 10, 20, 24, and 33 µM, respectively (11). Since cAK belongs to a large protein kinase family, it is possible that there are other high-affinity sites of action for these compounds to exert their inhibitory activities. Hence, *in vivo* biological activity and insecticidal activity of *D. scandens* might be due to the potent inhibitory action of warangalone and robustic acid on cAK (11).

*D. scandens* is said to be one of the most commonly

used medicinal plants in Thailand (4). Rural people usually take infusion of roasted dried stem of *D. scandens* or alcoholic macerate of *D. scandens* stem for the relief of muscle ache after work or as tonic. Therefore, it is interesting to study the effect of *D. scandens* on the immune system in order to determine if this medicinal plant possesses immunomodulating activity and has a potential to be used as health food to boost the immune system. Effects of 50% ethanolic extract of *D. scandens in vitro* on lymphocyte proliferation, T-cell mitogen response, interleukin-2 release and natural killer (NK) activity were studied using mouse splenic lymphocytes. In addition, the effect of this extract given orally in mice for 15 days on lymphoproliferation and mitogen response will also be examined.

## METHODS

*Plant material and preparation of plant extract:* Dried stem of *D. scandens* was obtained from Chao Praya Apaipubate Hospital, Pracheenburi province. The plant was identified by Miss Supaporn Pitiporn, the head pharmacist of the hospital. 50% Ethanolic extract of *D. scandens* was prepared by reflux method and the extract obtained was complexed with polyvinyl pyrrolidone (PVP) 30,000 at 1:2 ratio of extract to PVP in order to increase solubility of the extract.

*Animals:* Male Balb/c mice were bred and reared in specific pathogen free (SPF) condition in the animal facility of the National Institute of Health building, Department of Medical Sciences, Nonthaburi. The animals aged 6-8 weeks old weighing 25-30 g were used in this study.

*Cell line:* YAC-1 cell line, a lymphoma induced by inoculation of the Moloney leukemia virus into a newborn A/Sn mouse, was obtained from American Type Culture Collection (ATCC, Rockville, Maryland, USA). This cell line was used as target cells of mouse natural killer cells for the assay of the effect of *D. scandens* extract on NK activity.

*Preparation of suspension of mouse splenic lymphocytes:* Six male Balb/c mice were used in each set of experiment. Each animal was lightly anesthetized with ether and sacrificed by cervical dislocation. The spleen was aseptically removed, and dispersed in 5 ml of RPMI1640 media supplemented with 10% fetal bovine serum, penicillin 100 Units/ml, streptomycin 100 µg/ml, and glutamine 2 mM (complete media). Tissue debris was allowed to settle and the supernatant was removed and centrifuged at 1,000 rpm for 5 min at 4°C. RBC were lysed with Tris-$NH_4Cl$ solution and splenic lymphocytes were then washed three times with complete media before suspending in 5 ml complete media. Cell numbers and viability of spenic lymphocyte suspensions were determined using hemocytometer and trypan blue exclusion technique. Cell dilution was made with complete media to obtain required final cell concentration for each assay.

*Lymphocyte proliferation assay:* Effect of *D. scandens* extract on lymphocyte proliferation was determined by plating 100 ml of 2 x $10^6$ cells/ml of splenic lymphocytes from each mouse in triplicates in 96-well microtiter plates containing 50 µl of complete media and 50 µl of various dilutions of the extract prepared at 4x desired final concentrations. Control wells received 100 µl of 2 x $10^6$ cells/ml of splenic lymphocytes and 100 µl of complete media. Cells were cultured with the extract for 48 hours at 37°C, then were pulsed with 10 µl of 0.5 µCi [methyl-$^3$H]-thymidine (Amersham TRA120) per well. After 24-hour incubation, cells were harvested onto glass fiber filter and radioactivity was counted in a liquid scintillation counter (Wallac).

*Mitogen response:* Two types of T cell mitogen, phytohaemagglutinin (PHA) and concanavalin A (ConA) were used in this study. Similarly, effect of *D. scandens* extract on mitogen response of mouse splenic lymphocytes was determined by plating 100 µl of 2 x $10^6$ cells/ml of splenic lymphocytes in triplicates in 96-well microtiter plates containing 50 µl of mitogen solution and 50 µl of various dilutions of the extract prepared at 4x desired final concentrations. Suboptimal concentration was selected for each mitogen and final concentrations of PHA and ConA were 0.25 µg/ml and 0.2 µg/ml, respectively. Control wells received 100 µl of 2 x $10^6$ cells/ml of splenic lymphocytes and 50 µl of complete media and 50 µl of mitogen. Cells were cultured, pulsed with [$^3$H]-thymidine, harvested and counted for radioactivity as described in lymphoproliferation assay.

The activity of each concentration of the extract to

value = 1.39.

*ConA response:* The potentiating effect of the extract on ConA response of mouse splenic lymphocytes was observed at the extract concentrations of 0.78 - 25 µg/ml, with the maximum SI of 1.92 at the extract concentration of 12.5 µg/ml (Figure 3).

*Effect of the extract on IL-2 release:* Since the extract could stimulate lymphocyte proliferation and enhance mitogen response, it was then determined if it was due to the increase of IL-2 release. It was found that, without mitogen in the culture medium, the concentration of IL-2 released into the medium was below the detection limit of the IL-2 ELISA kit (data not shown); therefore, the effect of the extract on IL-2 release in lymphoproliferation assay could not be determined. However, in the presence of sub-optimal concentration of PHA, the extract at the concentration of 12.5, 25 and 50 µg/ml significantly increased IL-2 release from mouse splenic lymphocytes in a dose-dependent manner (Figure 4)

*Effect of the extract on NK activity:* The effect of various concentrations of the extract on spontaneous release of $^{51}Cr$ from $^{51}Cr$-loaded YAC-1 cells were initially tested. It was found that the extract at the concentrations of 1.56 µg/ml or lower did not affect spontaneous release of $^{51}Cr$ from YAC-1 cells, but the extract at the concentrations of 3.12 µg/ml or higher significantly increased spontaneous release of $^{51}Cr$ (data not shown). Hence, the effect of the extract at the concentrations of 1.56 µg/ml or lower were tested on NK activity. As shown in Figure 5, the extract at these concentrations did not significantly alter NK activity of mouse splenic lymphocytes either at the EC:TC ratio of 50:1 or 25:1.

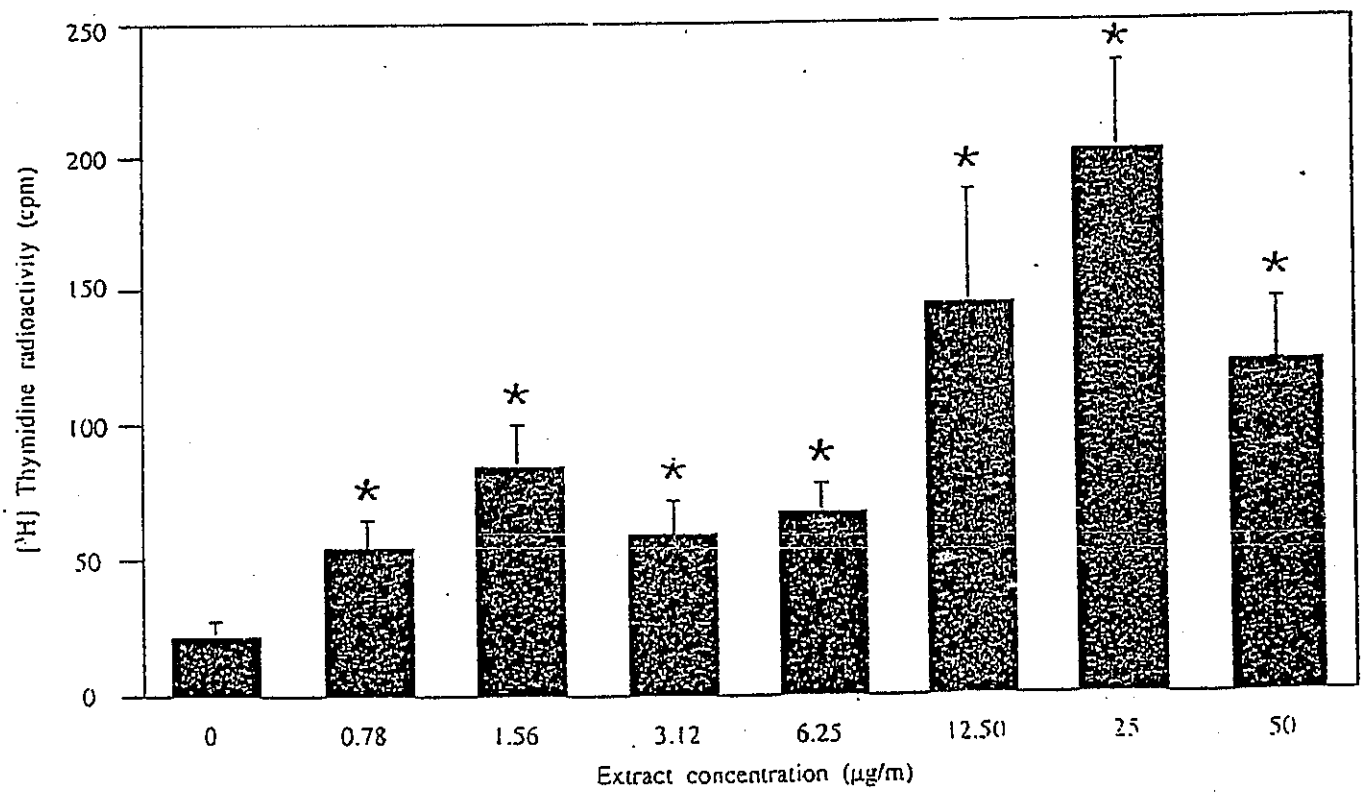


**Figure 1** *In vitro* effect of *D. scandens* on lymphoproliferation. Splenic lymphocytes (2x10⁵ cells/well/200 µl) from six mice were cultured in triplicate in medium containing no extract (control) or different concentrations of *D. scandens* extract for 48 hours before the addition of $^{3}H$-thymidine (0.5 mCi/well). Twenty-four hours later, cells were harvested onto glass fiber filter and the radioactivity of each well was counted. Each bar represents mean cpm ± SD (n=6).

* Significantly different from control ($p<0.05$).

increase lymphocyte proliferation or enhance mitogen response could also be expressed as *Stimulation Index (SI)* which was calculated as average cpm of lymphocytes incubated with extract divided by average cpm of control.

*Interleukin-2 assay:* The effect of the extract on the release of IL-2 was assayed by ELISA method. Mouse splenic lymphocytes (2 x $10^5$ cells) were cultured in 200 µl of medium containing PHA 0.25 µg/ml and the extract at the final concentration of 0, 12.5, 25 and 50 µg/ml for 72 hours. The microtiter plate was then centrifuged at 1,000 rpm for 5 min and 130 µl of supernatant were drawn and stored in another microtiter plate at -70°C until the time of the assay. The concentrations of IL-2 released into the supernatant in the presence or absence of the extract were assayed using mouse interleukin-2 ELISA kit (Intertest-2x™, Genzyme, USA).

*Assay of natural killer (NK) activity:* The assay was performed in triplicates in 96-well U-bottom microtiter plates. Initially, YAC-1 cells were loaded with $^{51}Cr$ by incubating in media containing sodium chromate (1mCi/ml, Amersham, USA) for 90 minutes at 37°C. $^{51}Cr$-YAC-1 cells were washed three times to remove excess $^{51}Cr$, then counted and adjusted to 2 x $10^6$ cells/ml. Serial dilutions of *D. scandens* extract were prepared at 4x required final concentrations while cell concentration of splenic lymphocytes was adjusted to 5 x $10^6$ cells/ml in complete media. Spontaneous release (SR) of $^{51}Cr$ from $^{51}Cr$-YAC-1 cells and the effect of *D. scandens* extract on spontaneous release of $^{51}Cr$ was determined by mixing 50 µl of $^{51}Cr$-YAC-1 with 150 µl of media, or with 100 µl of media and 50 µl of different concentrations of *D. scandens* extract, respectively. Total releasable count (TRC) of $^{51}Cr$ from $^{51}Cr$-YAC-1 cells was measured by incubating 50 µl of $^{51}Cr$-YAC-1 with 150 µl of 1 N HCl. Effect of *D. scandens* extract on splenic NK activity was determined by mixing 50 µl of $^{51}Cr$-YAC-1 with 50 µl of media (control) or extract (test) and 100 µl of 5 x $10^6$ or 2.5 x $10^6$ lymphocytes/ml to obtain effector cells to target cell ratio (EC:TC) of 50:1 or 25:1. Microtiter plates were centrifuged at 600 rpm for 3 minutes prior to incubation at 37°C for 4 hours. At the end of incubation period, the plates were centrifuged again at 1,000 rpm for 5 minutes and 100 µl of supernatant were removed from each well and counted for radioactivity of $^{51}Cr$ released into the media in a gamma counter (Packard, USA). %NK activity was calculated as follows :-

$$\%NK\ activity = \frac{CPM\ (control\ or\ test) - CPM\ (SR)}{CPM\ (TRC) - CPM\ (SR)} \times 100$$

*In vivo study: D. scandens* extract was given orally for 15 days to groups of 6 male Balb/c mice at the doses of 6.72 and 672.3 mg/kg BW/day which was equivalent to crude drug 0.03 and 3 g/kg/day or 1 and 100 times of therapeutic dose, respectively. The control groups received PVP solution at the concentrations corresponding to those present in the extract complex. At the end of treatment period, the animals were sacrificed and splenic lymphocytes suspensions were prepared at the cell concentration of 2 x $10^6$ cells/ml. Lymphocytes from both groups were cultured in complete media or in different concentrations of PHA (0.25, 0.5 & 1.0 µg/ml) and ConA (0.1, 0.2 & 0.4 µg/ml) to determine *in vivo* effect of the extract on lymphoproliferation and on PHA response, respectively. Lymphocytes were cultured and treated as described in the *in vitro* experiments and the radioactivity of incorporated thymidine of the extract-treated groups will then be compared with those of the control groups.

*Statistical analysis:* Average cpm of each triplicate sample was calculated. In *in vitro* experiment, cpm of wells containing extract was compared with cpm of control wells without extract using paired t-test, while unpaired t-test was used to compare cpm of control and treatment groups in *in vivo* study. Significant difference was set at $p<0.05$.

## RESULTS

In vitro *studies*

*Effect of the extract on lymphoproliferation:* As shown in Figure 1, it was found that the extract at the concentrations of 0.78 – 50 µg/ml significantly increased proliferation of mouse splenic lymphocytes as compared to those incubated in the absence of the extract ($p<0.05$). The extract at the concentration of 25 µg/ml gave maximum stimulation with SI value = 8.86.

*Effect of the extract on T- cell mitogen response:*

*PHA response:* Similarly, the extract at the concentrations of 0.78, 1.56, 12.5, 25 and 50 µg/ml significantly enhanced PHA response of mouse splenic lymphocyte (Figure 2). The extract at the concentration of 50 µg/ml gave maximum enhancement of PHA response with SI

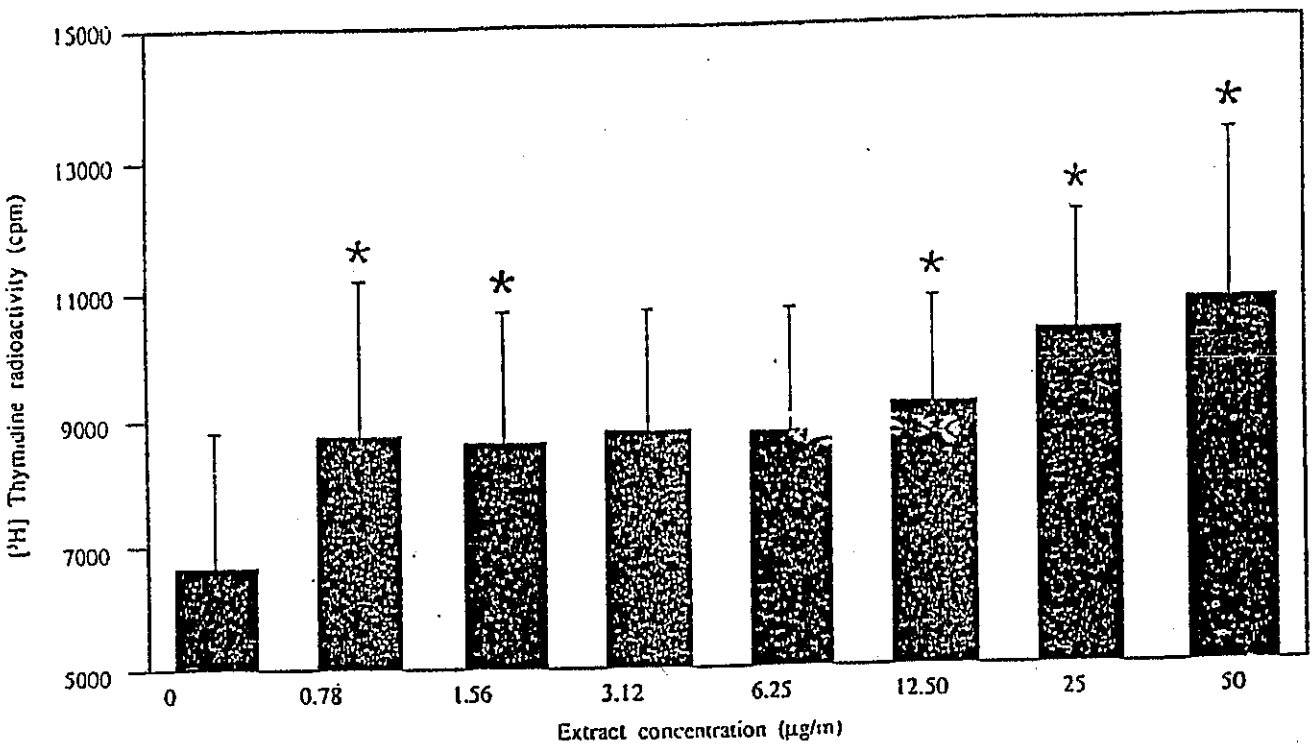


Figure 2 *In vitro* effect of *D. scandens* extract on PHA response. Splenic lymphocytes ($2x10^5$ cells/well/200 ml) from six mice were cultured in triplicate in medium containing PHA (0.25 µg/ml) with no extract (control) or different concentrations of *D. scandens* extract for 48 hours before the addition of $^3$H-thymidine (0.5 µCi/well). Twenty-four hours later, cells were harvested onto glass fiber filter and the radioactivity of each well was counted.
Each bar represents mean cpm ± SD (n=6).
* Significantly different from control ($p<0.05$).

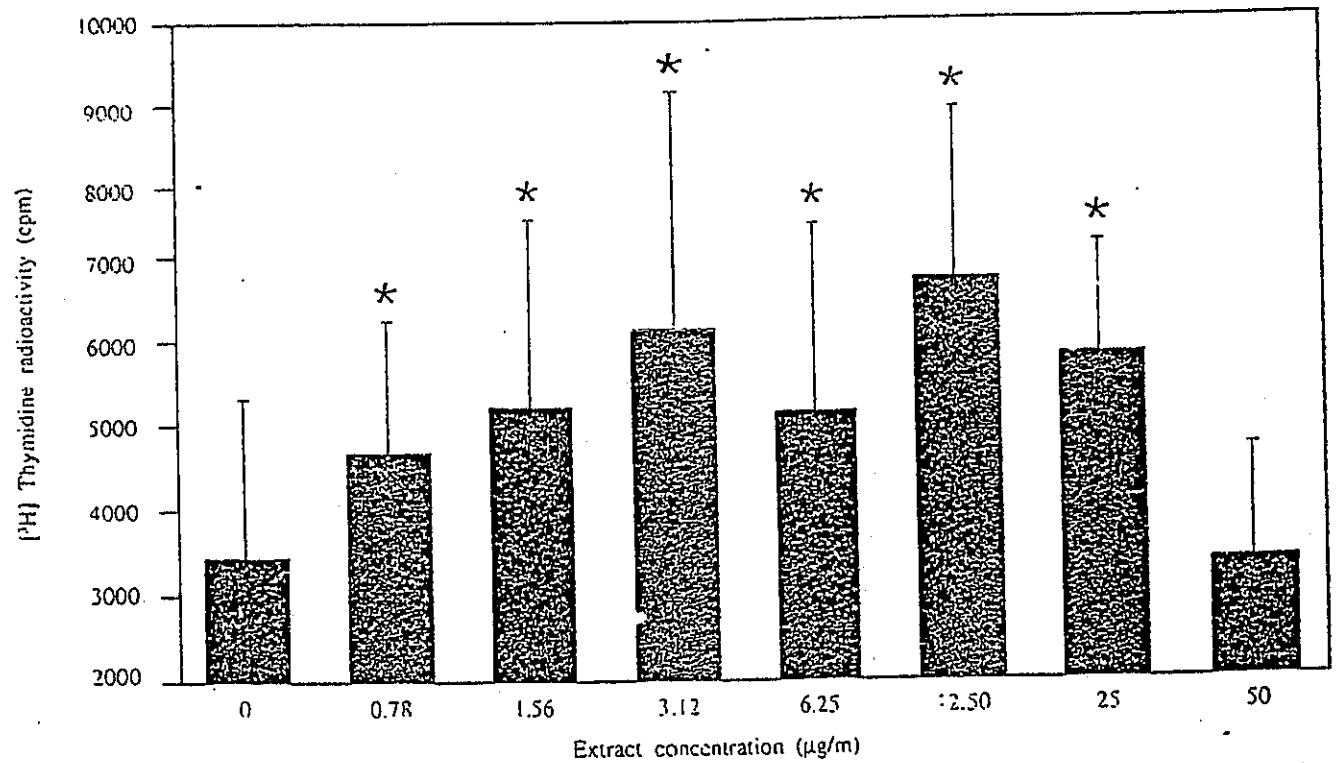


Figure 3 *In vitro* effect of *D. scandens* extract on ConA response. Splenic lymphocytes ($2x10^5$ cells/well/200 µl) from six mice were cultured in triplicate in medium containing ConA (0.2 µg/ml) with no extract (control) or different concentrations of *D. scandens* extract for 48 hours before the addition of $^3$H-thymidine (0.5 µCi/well). Twenty-four hours later, cells were harvested onto glass fiber filter and the radioactivity of each well was counted.
Each bar represents mean cpm ± SD (n=6).
Significantly different from control ($p<0.05$).

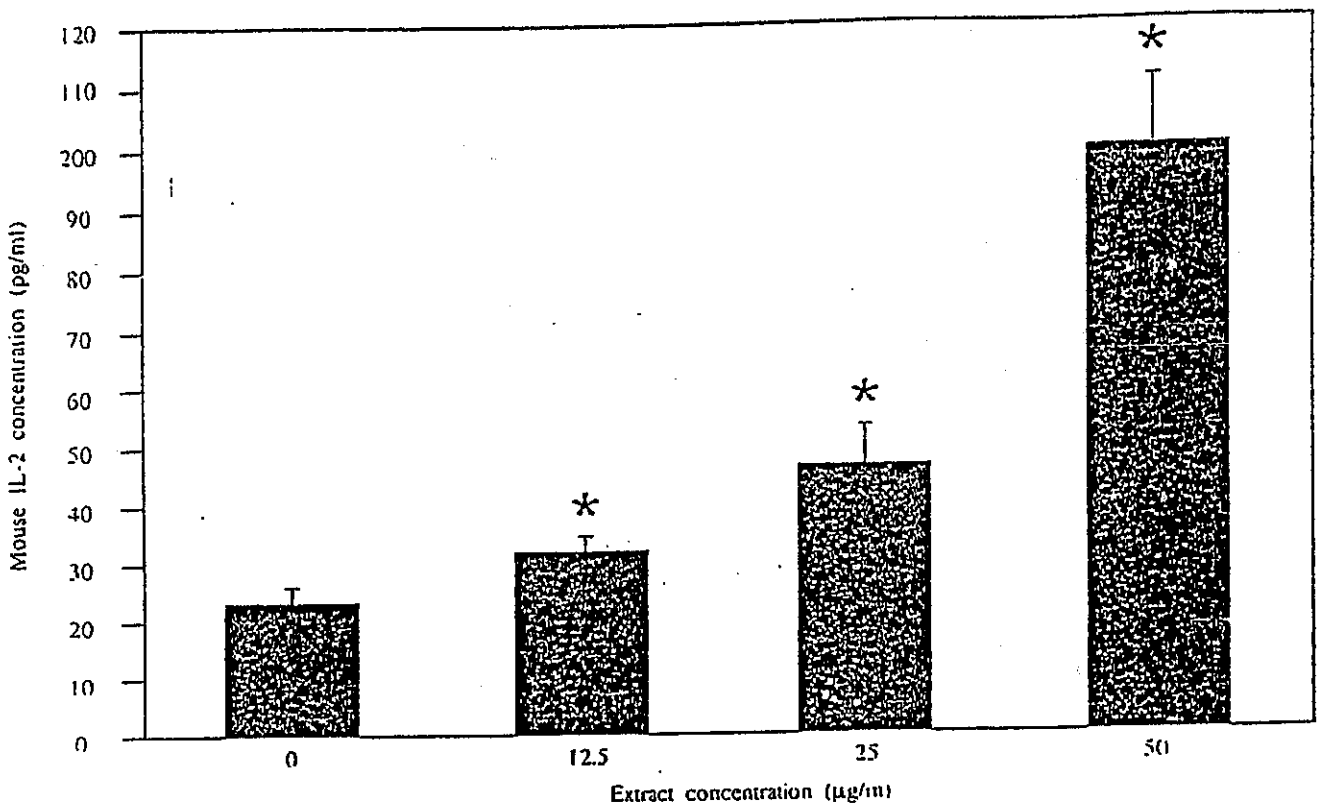


Figure 4 *In vitro* effect of *D. scandens* extract on IL-2 release. Splenic lymphocytes ($2x10^5$ cells/well/200 µl) from six mice were cultured in triplicate in medium containing PHA (0.25 µg/ml) with no extract (control) or different concentrations of *D. scandens* extract for 72 hours. After centrifugation, culture medium was removed for IL-2 assay by ELISA method. Each bar represents mean ± SD (n=6).
Significantly different from control ($p<0.05$).

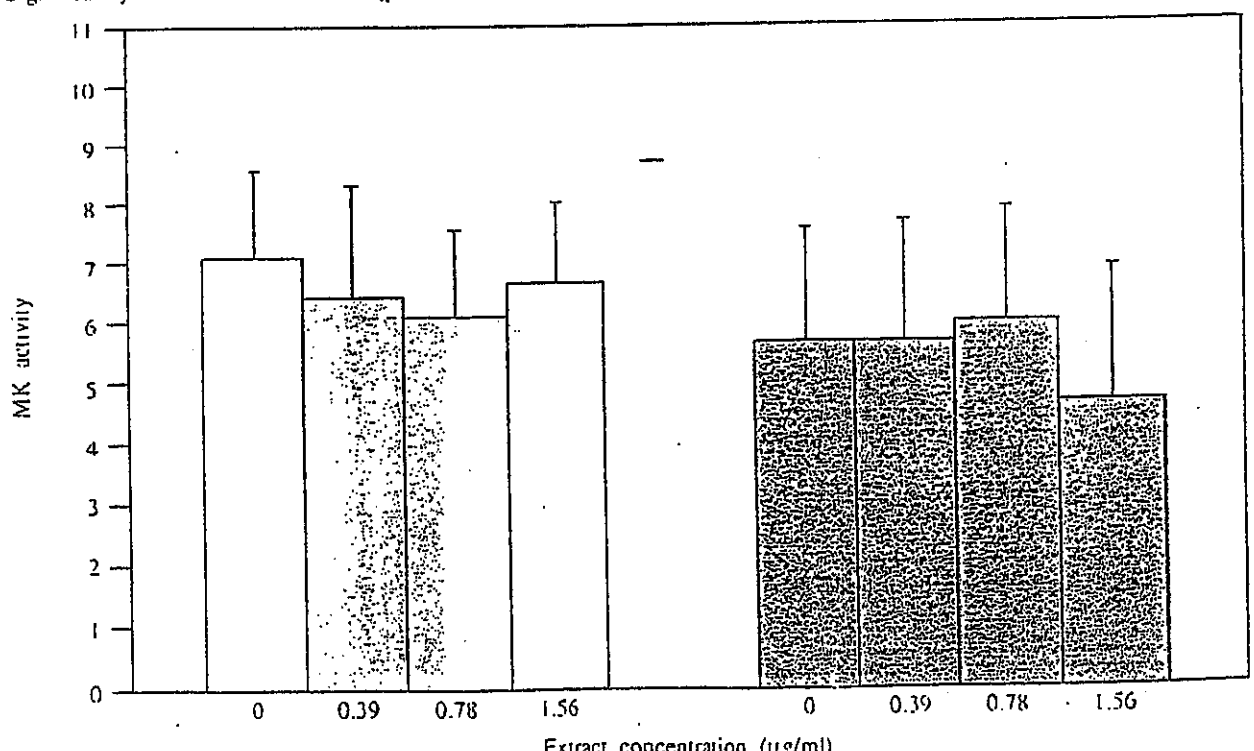


Figure 5 *In vitro* effect of *D. scandens* on NK activity. Splenic lymphocytes ($2.5x10^5$ and $5x10^5$ cells/well/200 µl) from 6 mice were cultured in triplicate with $^{51}$Cr-YAC-1 ($1x10^4$ cells/well) in the presence or absence of different concentrations of *D. scandens* extract at 37°C for 4 hours. After brief centrifugation, 100 ml of supernatant from each well were removed, counted for radioactivity, and calculated for %NK activity. %NK activity of wells containing extract was compared with those containing no extract (control).
Each bar represents mean ± SD. (n=6).

*In vivo study*

In vivo *effect of the extract on lymphoproliferation or mitogen response:* Mice were given either low dose (6.72 mg/kg BW) or high dose (672 mg/kg BW) of the extract which was equivalent to crude drug 0.03 or 3 g/kg-BW, or 1 to 100 folds of therapeutic dose, for 15 days. At the end of the treatment period, mouse splenic lymphocytes were cultured without the presence of mitogen or with different concentrations of PHA and ConA. It was found that in the absence of mitogen, lymphoproliferation of splenic lymphocytes of treatment groups, either low- or high-dose groups, was not different from that of the control groups (Table 1-4). Similarly, PHA responses of splenic lymphocytes from low-dose and high-dose groups were not significantly different from those of the controls regardless of the concentrations of PHA (Table 1 and 3). Likewise, proliferation of splenic lymphocytes from both low-dose and high-dose groups in response to different concentrations of ConA was not significantly different from those of the control groups (Table 2 and 4).

Table 1 *In vivo* effect of low dose of *D. scandens* extract on PHA response of splenic lymphocytes

| PHA concentration (μg/ml) | [$^3$H]-thymidine radioactivity (cpm) | | p value |
|---|---|---|---|
| | Control | *D. scandens* low dose | |
| 0 | 36.5 ± 11.2 | 40.5 ± 11.9 | 0.557 |
| 0.25 | 587.7 ± 185.6 | 1,185.7 ± 953.5 | 0.162 |
| 0.5 | 10,693.5 ± 2,139.5 | 16,081.6 ± 6,356.4 | 0.077 |
| 1.0 | 26,503.6 ± 8,165.9 | 35,832.5 ± 18,726.9 | 0.289 |

Balb/c mice were divided into 2 groups of six. Control group received PVP solution 13.4 mg/kg BW while *D. scandens* low dose group received the extract 6.72 mg/kg BW which was equivalent to crude drug 0.03 g/kg BW for 15 days. The animals were then sacrificed and splenic lymphocyte suspensions were prepared. Lymphocytes ($2x10^5$ cells/well/200 μl) were cultured in the presence or absence of different concentrations of PHA for 48 hours before the addition of [$^3$H]-thymidine (0.5 μCi/well). Twenty-four hours later, cells were harvested onto glass fiber filter and the radioactivity of each well was counted.

Each value represents mean ± SD (n=6).

Table 2 *In vivo* effect of low dose of *D. scandens* extract on ConA response of splenic lymphocytes

| ConA concentration (μg/ml) | [$^3$H]-thymidine radioactivity (cpm) | | p value |
|---|---|---|---|
| | Control | *D. scandens* low dose | |
| 0 | 37.5 ± 5.4 | 40.9 ± 13.8 | 0.582 |
| 0.1 | 85.6 ± 29.3 | 213.2 ± 165.0 | 0.092 |
| 0.2 | 944.7 ± 485.1 | 2,201.3 ± 1,925.4 | 0.152 |
| 0.4 | 4,474.0 ± 4,169.2 | 8,766.4 ± 5,385.3 | 0.154 |

Balb/c mice were divided into 2 groups of six. Control group received PVP solution 13.4 mg/kg BW while *D. scandens* low dose group received the extract 6.72 mg/kg BW which was equivalent to crude drug 0.03 g/kg BW for 15 days. The animals were then sacrificed and splenic lymphocyte suspensions were prepared. Lymphocytes ($2x10^5$ cells/well/200 μl) were cultured in the presence or absence of different concentrations of ConA for 48 hours before the addition of [$^3$H]-thymidine (0.5 mCi/well). Twenty-four hours later, cells were harvested onto glass fiber filter and the radioactivity of each well was counted.

Each value represents mean ± SD (n=6).

Table 3 *In vivo* effect of high dose of *D. scandens* extract on PHA response of splenic lymphocytes

| PHA concentration (μg/ml) | [³H]-thymidine radioactivity (cpm) | | p value |
|---|---|---|---|
| | Control | *D. scandens* low dose | |
| 0 | 93.7 ± 59.8 | 91.7 ± 31.5 | 0.942 |
| 0.25 | 1,311.6 ± 790.5 | 2,244.6 ± 1,151.9 | 0.210 |
| 0.5 | 13,212.1 ± 4,211.8 | 15,847.1 ± 5,846.1 | 0.391 |
| 1.0 | 22,681.3 ± 7,796.2 | 23,631.7 ± 8,792.4 | 0.847 |

Balb/c mice were divided into 2 groups of six. Control group received PVP solution 1.34 g/kg BW while *D. scandens* high dose group received the extract 0.672 g/kg BW which was equivalent to crude drug 3.0 g/kg BW for 15 days. The animals were then sacrificed and splenic lymphocyte suspensions were prepared. Lymphocytes ($2x10^5$ cells/well/200 μl) were cultured in the presence or absence of different concentrations of PHA for 48 hours before the addition of [³H]-thymidine (0.5 μCi/well). Twenty-four hours later, cells were harvested onto glass fiber filter and the radioactivity of each well was counted.

Each value represents mean ± SD (n=6).

Table 4 *In vivo* effect of high dose of *D. scandens* extract on ConA response of splenic lymphocytes

| ConA concentration (μg/ml) | [³H]-thymidine radioactivity (cpm) | | p value |
|---|---|---|---|
| | Control | *D. scandens* low dose | |
| 0 | 123.3 ± 36.0 | 123.8 ± 29.9 | 0.980 |
| 0.1 | 914.9 ± 801.2 | 1,449.2 ± 1,536.4 | 0.467 |
| 0.2 | 6,008.2 ± 4,468.7 | 7,228.8 ± 4,823.6 | 0.659 |
| 0.4 | 15,958.1 ± 7,827.1 | 17,429.0 ± 9,739.5 | 0.778 |

Balb/c mice were divided into 2 groups of six. Control group received PVP solution 1.34 g/kg BW while *D. scandens* high dose group received the extract 0.672 g/kg BW which was equivalent to crude drug 3.0 g/kg BW for 15 days. The animals were then sacrificed and splenic lymphocyte suspensions were prepared. Lymphocytes ($2x10^5$ cells/well/200 μl) were cultured in the presence or absence of different concentrations of ConA for 48 hours before the addition of [³H]-thymidine (0.5 μCi/well). Twenty-four hours later, cells were harvested onto glass fiber filter and the radioactivity of each well was counted.

Each value represents mean ± SD (n=6).

## DISCUSSION

During the past several years, people all over the world have become interested in the use of natural products in place of pure chemicals as medicines and health products. Several medicinal plants and natural products have been studied for their immunomodulating activity in an attempt to find medicinal plants that help strengthening the immune system to fight against diseases, e.g. *Picrorhiza kurroa* (12,13), *Azadirachta indica*, *Jatropha multifida* (13), *Andrographis paniculata* (14), *Helleborus niger* (15), *Chlorella pyrenoidosa* (16), etc.

*D. scandens* is said to be one of the most commonly used medicinal plants in Thailand especially in the rural areas of the country (4). It is commonly used in the form of alcoholic macerate as tonic and to relieve muscle ache and pain. Hence, it is interesting to study its alcoholic extract on cell-mediated immunity and certain parameter of innate immunity, e.g. NK activity.

In *in vitro* studies, it was found that the extract at the concentrations of 0.78-50 μg/ml could stimulate lymphoproliferation of mouse splenic lymphocytes with

the maximal SI of 8.86 at 25 μg/ml (Figure 1). Moreover, the extract could also enhance PHA and ConA response (Figure 2, 3) but did not alter NK activity of mouse splenic lymphocytes (Figure 5). The results suggested that the extract might contain an active constituent that could act as a mitogen to stimulate lymphocyte proliferation and enhance T cell mitogen response. As shown in Figures 1 and 3, the stimulatory effect of the extract at low concentrations on lymphoproliferation and ConA response showed some dose-response relationship until the concentration of the extract was higher than the optimal concentration where stimulatory activity started to decline. This type of dose-response relationship was commonly observed for lymphocyte proliferation under mitogen stimulation (17). Taken together, the results suggested that the stem of *D. scandens* contained some ethanol-soluble substances that could stimulate lymphoproliferation and T-cell mitogen response. The isolation and elucidation of the structure of the active compound stimulating lymphocyte proliferation remain to be further investigated.

Since IL-2 is the major T-cell cytokine involved in T cell proliferation, we determined if lymphocyte proliferation and enhanced mitogen response induced by the extract was due to the stimulation of IL-2 release. Concentrations of IL-2 in the culture medium in the absence and presence of the extract were assayed by ELISA technique. Without mitogen in the culture medium, the amount of IL-2 released into the medium was below the detection limit of the IL-2 ELISA kit; therefore, it was not known whether lymphoproliferative effect of the extract was due to the increase of IL-2 release or not. However, in the presence of sub-optimal concentration of PHA, it was found that the extract increased the release of IL-2 into the culture medium in a dose-response manner (Figure 4). The result suggested that the enhancement of mitogen response *in vitro* be, at least in part, due to the stimulatory effect of the extract on IL-2 release.

Warangalone, robustic acid and prenylisoflavone derivatives present in *D. scandens* were reported to be selective and potent inhibitors of rat liver cyclic AMP-dependent protein kinase catalytic subunit (11). Hence, it is possible that there are other high-affinity sites of action for these compounds, e.g. in lymphocytes. Human T lymphocyte was found to contain two isozymes of cyclic AMP-dependent protein kinase (18). It was reported that cyclic AMP-dependent protein kinase type I mediated the inhibitory effects of cAMP on cell replication in human T lymphocytes (19). Hence, the stimulatory activity of the extract on mouse splenic lymphocyte proliferation and mitogen response may also involve the inhibitory effect on lymphocyte cAMP-dependent protein kinase catalytic subunit by some active principles of *D. scandens* present in the extract.

Since *D. scandens* extract could enhance lymphoproliferation and mitogen response *in vitro*, we then examined if the extract, given orally at the doses equivalent to 1 and 100 folds of therapeutic dose for 15 days, would exhibit similar effects *in vivo*. As shown in Table 1-4, in the absence of PHA or ConA in the culture medium, the proliferation of splenic lymphocytes from the animals in low- or high-dose groups was not different from that of the control groups. In the presence of mitogen in the culture medium, even though the proliferation of lymphocytes from extract-treated groups appeared to be higher than that of the control groups especially at the low concentration of mitogen, the difference was not statistically significant.

The apparent lack of the *in vivo* immunomodulatory effect of the extract could be due to several reasons. The dose of the extract might not be high enough to exert any stimulatory effect or the active principle in the extract might not be absorbed from the GI tract. In contrast, the active principle may be absorbed but was metabolized to an inactive compound or was rapidly excreted from the body.

Another possibility is that the extract may possess *in vivo* immunostimulatory effect on mitogen response but this effect could not be detected due to the variability of mitogen response of splenic lymphocytes from each individual animal. This individual variation makes it more difficult to detect subtle difference between treatment and control groups. Unfortunately, it is practically impossible to compare mitogen response of splenic lymphocytes before and after receiving the extract, or within group comparison, which should eliminate the problem of individual variation because each animal will serve as its own control.

Even though *D. scandens* extract did not show

immunostimulatory activity *in vivo* in mice, its significant *in vitro* activity still warrants further investigation in humans. The Medicinal Plant Research Institute has just finished subchronic toxicity study of 50% ethanolic extract of *D. scandens* in rats and histopathological examination of internal organs is currently underway. After the results of the toxicity study is concluded and the extract is found to be safe, it is interesting to study immunomodulatory activity of the extract in humans by comparing lymphoproliferation and mitogen response of peripheral blood mononuclear cells before and after receiving the extract. If the extract shows immunostimulatory activity, this plant will have the potential to be used to boost the immune system in humans, e.g. in AIDS and cancer patients.

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# การศึกษาฤทธิ์กระตุ้นภูมิคุ้มกันของเถาวัลย์เปรียง

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บทคัดย่อ : เถาวัลย์เปรียง *(Derris scandens Benth.)* เป็นสมุนไพรที่นิยมใช้กันมากที่สุดชนิดหนึ่งในประเทศไทย ตามตำรายาไทยสำต้นของเถาวัลย์เปรียงมีสรรพคุณในการบรรเทาอาการปวดเมื่อยกล้ามเนื้อ ขับปัสสาวะ ขับเสมหะ แก้ไอ และเป็นยาบำรุง จึงได้นำสารสกัดด้วย 50% เอธานอลของเถาวัลย์เปรียงมาศึกษาฤทธิ์ต่อระบบภูมิคุ้มกันผ่านเซลล์ (cell-mediated immunity) โดยศึกษาผลของสารสกัดในหลอดทดลอง (*in vitro* activity) ต่อลิมโฟซัยท์จากม้ามของหนูถีบจักร ได้แก่ ผลต่อการแบ่งตัวของลิมโฟซัยท์ (lymphoproliferation) การตอบสนองต่อฤทธิ์กระตุ้นให้เซลล์แบ่งตัวของสารจำพวก mitogens และผลต่อ activity ของ natural killer cells (NK activity) พบว่า สารสกัดในช่วงความเข้มข้น 0.78-50 µg/ml สามารถกระตุ้นการแบ่งตัวของลิมโฟซัยท์ รวมทั้งสามารถเสริมฤทธิ์ของ T-cell mitogens ได้แก่ phytohaemagglutinin (PHA) และ concanavalin A (conA) ในการกระตุ้นให้ลิมโฟซัยท์แบ่งตัวได้ การเสริมฤทธิ์ของ T-cell mitogen นี้อาจเนื่องมาจากฤทธิ์กระตุ้นหลั่ง interleukin-2 ของสารสกัด อย่างไรก็ตามสารสกัดในขนาด 0.39-1.56 µg/ml ไม่มีผลต่อ NK activity เนื่องจากสารสกัดของเถาวัลย์เปรียงแสดงฤทธิ์กระตุ้นการแบ่งตัวของลิมโฟซัยท์ในหลอดทดลอง จึงได้ศึกษาผลของสารสกัดที่ให้ทางปากแก่หนูถีบจักร (*in vivo* activity) นาน 15 วัน ในขนาดเทียบเท่าผงยา 0.03 และ 3 กรัมต่อน้ำหนักตัว 1 กิโลกรัม (ก./กก./วัน) หรือคิดเป็น 1 และ 100 เท่าของขนาดที่ใช้ในคนเทียบกับหนูกลุ่มควบคุม พบว่าการแบ่งตัวในสภาวะที่ไม่มี mitogen ของลิมโฟซัยท์จากม้ามของหนูถีบจักรที่ได้รับสารสกัดทั้งสองขนาดไม่แตกต่างจากของหนูกลุ่มควบคุมอย่างมีนัยสำคัญทางสถิติ ส่วนการตอบสนองต่อฤทธิ์กระตุ้นให้ลิมโฟซัยท์แบ่งตัวของ PHA ขนาด 0.25-1 µg/ml หรือ ConA ขนาด 0.1-0.4 µg/ml ของหนูที่ได้รับสารสกัดทั้งสองขนาดก็ไม่แตกต่างจากกลุ่มควบคุมอย่างมีนัยสำคัญทางสถิติเช่นกัน จากผลการทดลองสรุปได้ว่าสารสกัดด้วย 50% เอธานอลของเถาวัลย์เปรียงสามารถแสดง *in vitro* immunomodulating activity อย่างมีนัยสำคัญต่อลิมโฟซัยท์จากม้ามของหนูถีบจักร อย่างไรก็ตามเมื่อให้สารสกัดทางปากในขนาด 1 เท่าและ 100 เท่าของขนาดที่ใช้ในคนเป็นเวลา 15 วัน พบว่าการแบ่งตัวของลิมโฟซัยท์ทั้งในสภาวะที่มีและไม่มี T-cell mitogen ไม่แตกต่างจากกลุ่มควบคุมอย่างมีนัยสำคัญทางสถิติ ซึ่งความแตกต่างของผลการทดลองทั้งสองส่วนนี้อาจมีได้หลายสาเหตุ

กุญแจคำ : เถาวัลย์เปรียง, ระบบภูมิคุ้มกัน, ลิมโฟซัยท์, อินเตอร์ลูคิน-2