The *Phyllanthus emblica* L. Infusion Carries Immunostimulatory Activity in a Mouse Model

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Background: *Phyllanthus emblica L. (Indian gooseberry, Ma khaam pom) has been an herbal component of Thai traditional recipes proposed to slow down the aging process. A number of methodologies have been employed to investigate the immunological aspects of the so called "anti-aging effects" of P. emblica in a BALB/c mice model.*
Objective: *1) To investigate the immunological efficacy of the anti-aging effects of P. emblica infusion in a BALB/c mice model. 2) To verify the safety for the consumption of P. emblica infusion in BALB/c mice.*
Material and Method: *For in vitro studies, splenocytes were isolated from mice and examined in comparison with the human umbilical endothelial cells, fibroblasts and YAC-1 (mouse lymphoma) cells for proliferative activity upon the exposure to P. emblica infusion. For in vivo studies, mice were orally administered with P. emblica infusion at a dose range of 0, 50, 100, 200 mg/kg BW for 14 days. After the treatments, splenocytes isolated from these mice examined for proliferative and NK cell activities.*
Results: *For in vitro studies, the infusion of P. emblica could directly drive the proliferation of mouse splenocytes in a dose-dependent manner. The P. emblica infusion itself was already cytotoxic to YAC-1 in the studied dose, while sparing the human umbilical endothelial cells and fibroblasts. For in vivo studies, splenocytes isolated from these mice exhibited dose-dependent proliferative activities. Only the isolated splenocytes from mice ingesting 100 mg/kg BW exhibited an enhancement in NK cell activity.*
Conclusion: *P. emblica infusion could drive proliferative activity of splenocyte in vitro and in vivo, with an enhancement in the NK cell-induced cytotoxic activity. The infusion in the aforementioned dose was safe throughout the study.*

Keywords: *Phyllanthus emblica, Splenocyte, Immunostimulation*

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Health concern has been shifting toward improving conditions of longevity. Medicinal plants and herbal recipes that have been traditionally prescribed for longevity might already provide the solution. However, the components of the so called "longevity" have not been clearly defined. None of the medicinal plants/ herbal recipes has been scientifically confirmed for longevity. The authors postulated that the immunomodulatory activity is a major component of longevity and chose *Phyllanthus emblica* as a candidate for this test based on its traditional prescription. *P. emblica* Linn. (Indian gooseberry, Ma khaam pom) is a plant in the family of Euphorbiaceae indigenous in Thailand and has been known as a rich source of vitamin C. Existing published data have reported its diverse therapeutic effects including antimicrobial[1], analgesic/antipyretic[2], anti-inflammation[2,3], antioxidant[4-7], antimutagenic[8] and anticancer[9].

The anticancer activity of *P. emblica* has been demonstrated[9-11]. *P. emblica* extract inhibited the proliferation of a variety of tumor cell lines *in vitro* such as L929 cells[9], human leukemic (HK63) cells[8], MDA-MB-231 cells[12], Hela cells[12], MCF-7[11]. The

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synergistic effect with chemotherapeutic agents has been reported for hepatocellular carcinoma and lung cancer cells[13]. The aqueous extract of the fruit was cytotoxic to L929 cells and was able to reduce tumor ascites in mice induced by DLA cells. The aqueous extract did not generate acute or chronic toxicity toward Wistar rat[14], but induced apoptosis in pancreatic tumor xenograft[15]. It increased life span of tumor-bearing mice and effectively reduced tumor volume[9]. *P. emblica* has been applied as a component of combination therapy to alleviate the toxicity of chemotherapeutic agents[16].

A number of compounds isolated from different parts of this plant were determined as active components, especially compounds with a galloyl or pyrogallol group[9]. The extracts of *P. emblica* significantly inhibited hepatocarcinogenesis induced by N-nitrosodiethylamine in animals[17]. The fruits of *P. emblica* alleviated the immunosuppressive effects of chromium on lymphocyte proliferation and restored the production of interleukin 2 and interferon-γ[18].

Despite many effects of *P. emblica*, relatively little data are available on the putative immuno-modulatory effects of *P. emblica* on *in vitro* lymphocyte function. The authors proposed that the infusion of *P. emblica* contains immunostimulatory activity that indirectly served its anticancer action. A panel of *in vivo* and *in vitro* studies were undertaken to determine the immunomodulatory effects of *P. emblica* infusion using splenocytes from BALB/c mice as a model. Our sensitive methodologies would help clarifying whether *P. emblica* is efficacious toward immunostimulation. To verify this action, the therapeutic and toxic concentrations of infusion were determined directly on isolated primary mouse splenocytes and tumor cells as an *in vitro* study. For *in vivo* study, splenocytes were isolated from mice orally-fed with *P. emblica* infusion for 14 days and immediately examined for innate immunological activity. The outcomes from the present study would provide the safety/toxicity profile of *P. emblica* infusion and confirm the presence of immunomo-stimulatory effect of *P. emblica* infusion both *in vitro* and *in vivo*.

Material and Method

Preparation of Indian gooseberry (P. emblica L.) infusion

P. emblica infusion was manufactured by the Center of Applied Thai Traditional Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University. *P. emblica* (1 g/infusion bag) was boiled in 100 mL sterile distilled water (10 mg/mL) at 80°C for 10 min. The infusion was filtered through the Whatman glass filter, followed by the 0.2 micron syringe filter. The infusion was lyophilized to powder form and kept frozen in vacuum desiccators until dispensing.

Animal studies

Inbred male BALB/c mice were used for *in vivo* studies and as a source of splenocytes for *in vitro* studies. The animals were supplied by the National Animal Laboratory Center, Mahidol University, Salaya, Nakhonpathom. Mice were 5-6 weeks of age, weighing 25-30 g. The study protocol had been approved by Siriraj Animal Care and Use Committee (SI-ACUC). The number of animals followed the guideline of animal studies[19] with at least 15 mice/category. The sample number was already beyond the recommended minimum of 4 animals/group. All mice were housed in individual ventilated cages (IVC, Technicplast, Italy) at 4 mice per cage with sterile saw wood bedding to minimize stress. Animal rooms were maintained under controlled housing conditions (23 ± 2°C, 40-70% humidity) with a 12:12 h light:dark cycle at the animal facility of the Department of Immunology, Faculty of Medicine Siriraj Hospital, Mahidol University. Animals had free access to laboratory chow (C.P. feed number 082, Thailand) and sterile distilled water *ad libitum* throughout the 7-day acclimatization period.

The estimated daily recommended consumption of the *P. emblica* infusion for human (1-4 bags) was multiplied by 7 for animal study[20], resulting in the daily dose range between 50-200 mg/kg body weight. Mice were dosed for 14 days with 50, 100, or 200 mg/kg of *P. emblica* infusion in 0.2 mL sterile distilled water via oral gavages. They were weighted daily and observed for mortality. On the final day of study, 24 h after the last dose, animals were sacrificed by cervical dislocation. The spleen was dissected to isolate splenocytes.

Splenocytes

Splenocytes were prepared from control or treated mice. The spleens were aseptically excised and placed in 15-mL centrifuge tube containing 10 mL RPMI-1640 serum free medium. Under a lamina flow hood, single cell suspension was prepared by grinding the spleen using a syringe with 21 gauge needle and pipetted in 10 mL RPMI-1640 without fetal bovine serum (FBS). The cell suspension was laid over a dis-continuous IsoPrep (Robbins Scientific Corporation. Sunnyvale, CA) density gradient and centrifuged at

800 g for 20 min at 25°C to isolate mononuclear cells. The ring containing lymphocytes was collected and washed twice with RPMI-1640 containing 10% FBS by centrifugation at 800 g, 15°C for 10 min. The viability of the isolated splenocyte determined by trypan blue exclusion test was greater than 95%.

Other cultured cells

YAC-1 (CLS Cell Lines Service, Eppelheim, Germany) was used as natural target cells for mouse NK cells. It was cultured in T-25 tissue culture flasks with RPMI-1640 medium containing 20% FBS, 100 IU/mL penicillin G, 100 mcg/mL streptomycin at 37°C in 5% CO_2. Human umbilical vein endothelial cell culture (HUVEC) was maintained in endothelial-SFM basal growth medium with L-glutamine and M199 medium at the ratio of 1:1 (vol:vol), 10% FBS, 100 IU/mL penicillin G and 100 mcg/mL streptomycin at 37°C, 5% CO_2. The fibroblasts were cultured with Dulbecco's Modified Eagle's medium (DMEM), 15% FBS, 100 IU/mL penicillin G, 100 mcg/mL streptomycin at 37°C, 5% CO_2. All cultured cells were grown to sub-confluence (70-80%) until use.

The in vitro dose escalation study

The *in vitro* toxicity of *P. emblica* infusion was determined in splenocytes, YAC-1, fibroblasts and HUVECs. Cells were diluted in their respective media and seeded on each well at a density of 1 x 10^5 cells/well of a 96-well plate 24 h before experiment. Aliquots (100 mcL) of *P. emblica* infusion (0, 1, 3, 10, 30 and 300 mcg/mL) in RPMI, DMEM or M199 with 10% FBS were added to the wells. The splenocytes may be treated with lipopolysaccharide (LPS, 5 mcg/mL) or concanavalin A (ConA, 0.5 mcg/mL) to magnify cell numbers. Most cells were incubated at 37°C, 5% CO_2 for 72 h except HUVECs with only 24 h. Cell proliferation/viability was determined using the MTT colorimetric assay.

Splenocyte proliferation assay

Aliquots (200 mcL) of splenocytes in RPMI-1640, 10% FBS were plated at a density of 1 x 10^5 ells per well in a 96-well plate. A mitogen (0.5 mcg/mL ConA or 5 mcg/mL LPS) was added to each triplicate wells. The cells were incubated at 37°C and 5% CO_2 for 72 h. After the incubation, the plate was centrifuged at 800 g for 15 min to discard the medium. The MTT in serum-free serum (200 mcL of 2 mg/mL) was added to each well and incubated at 37°C, 5% CO_2 for 1 h. After which time, the medium was discarded. The intracellular MTT-formazan was solubilized with 100 mcL dimethyl-sulfoxide (DMSO). The plate was left at room temperature with an occasional gentle shaking for 5 min. The absorbance at 595 nm was measured using an ELISA microplate reader (SpectraMax M5, Molecular Devices). The absorbance of treated wells was calculated as the percentage of the control as follows:

$$\text{Proliferation (\%)} = \frac{\text{OD sample - OD control}}{\text{OD control}} \times 100$$

Natural Killer (NK) cell cytotoxic assay

Splenocytes were evaluated for natural killer (NK) cell cytotoxic activity using an ELISA microplate reader. As target cells, the YAC-1 (50 mcL of 5 x 10^3 cells/mL) was seeded in triplicate onto the flat-bottom 96-well plate. Subsequently, 50 mcL of effector cells was added to each well in triplicate, resulting in effector: target (E:T) ratios of 3.125:1 and 6.25:1 and 12.5:1. After incubation for 2.5 h at 37°C in 5% CO_2, 15 mcL of propidium iodide solution (20 mcg/mL in PBS) was added and incubated for additional 1.5 h. Fluorescence unit was measured using a 482 nm exciter filter and 630 nm emission filter. The mean fluorescence unit was obtained from triplicate wells and the cytotoxicity percentage was calculated as follows:

$$\text{Cytotoxicity (\%)} = \frac{\text{Test units- spontaneous units of target and effector}}{\text{Maximal units of target-spontaneous unis of target}} \times 100$$

Statistical analysis

The data were expressed as mean $\pm$ SEM and were analyzed using GraphPad Prism version 5.02. Either one-way ANOVA with Dunnett's test or two-way ANOVA with Bonferroni test was used to analyze statistical significance of the differences between the controls and the treated samples. Data were considered statistically significant if $p < 0.05$.

Results

The proliferation of splenocytes in response to P. emblica infusion

P. emblica infusion at 3 mcg/mL ($p < 0.05$) could significantly and directly raise splenocyte proliferation higher than those of the corresponding controls (0 mcg/mL) (Fig. 1). Addition of LPS slightly elevated the proliferative signal from the treatment at 30 and 300 mcg/mL ($p < 0.05$) over those of the corresponding controls. Addition of ConA provided little proliferative advantage to splenocytes. The addition of either mitogen (*i.e.*, LPS or ConA) did not exhibit any statistical significant difference from those without the addition. The *P. emblica* infusion masked

the stimulatory effect of all mitogens and produced a dose-dependent proliferative action.

The cytotoxicity study of P. emblica infusion

For YAC-1, the cytotoxicity *P. emblica* infusion (Fig. 2) started at lower doses (1 mcg/mL) while the toxicity to HUVEC and fibroblast was demonstrated at higher dose (100 mcg/mL). The inhibitory concentration at 50th percentile (IC50) was greater than 300 mcg/mL for both human fibroblast and HUVEC, but less than 10 mcg/mL for YAC-1.

The immunological profile of mice fed with P. emblica infusion

Mice fed with *P. emblica* infusion through oral gavage for 14 d had no difference in body weight from untreated mice throughout the study (data not shown). They were sacrificed and retrieved for splenocytes. Splenocytes from mice ingest*ing P. emblica* infusion at 100 mg/kg BW with ConA or without any mitogen and 50 and 100 mg/kg BW with LPS harbored significantly higher proliferation than those of the corresponding controls (0 mg/kg BW, Fig. 3). A dose-dependent elevation of proliferative effect was observed. For NK cell-derived cytotoxic activity, the significant cytotoxicity came from splenocytes isolated from mice ingesting 100 mg/kg *P. emblica* infusion at E:T ratio of 12.5:1 (Fig. 4).

Discussion

Constituents of P. emblica infusion

P. emblica is commercially available at the Center of Applied Thai Traditional Medicine as dried powder packaged in bag and consumed in infusion form. This commercial source was employed throughout this proposal and had acquired fingerprint analysis to confirm its integrity. TLC chromatogram and HPLC chromatogram indicated that our preparation of *P. emblica* infusion contained gallic acid as a measurable constituent. Since the preparation of aqueous extract employed hot water as the major solvent, this already explained the observation of hydrophilic substances (*i.e.*, gallic acid) as the major constituent[14]. Other reported constituents of *P. emblica* include tannins, flavonoids and other phenolic compounds[6]. The fruits contain low molecular weight tannoids, mainly emblicanins A and B, punigluconin, pedunculagin, polyphenolic substances such as gallic acid and ellagic acid[4-6,21]. The extraction of most of the aforementioned substances except gallic acid requires less polar solvents.

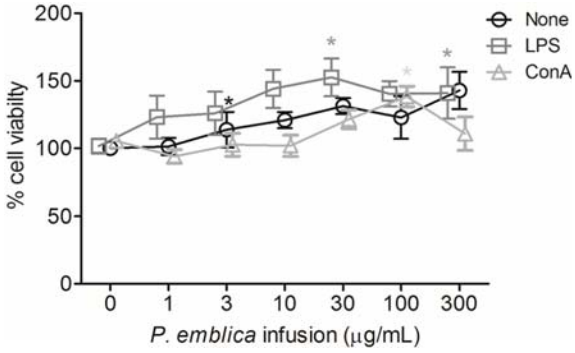

Fig. 1 The 72-h incubation of *P. emblica* infusion at 0, 1, 3, 10, 30, 100, 300, mcg/mL with splenocytes (1 x 10^5 cells/well). Splenocytes were stimulated either with LPS (5 mg/mL, □), ConA (0.5 mg/mL, △), or none (○) throughout the incubation. The proliferation of splenocytes was measured using MTT assay. Each value represented mean ± SEM of three independent experiments performed in triplicate (n = 9). Difference with statistical significance ($p < 0.05$) from the corresponding untreated control (0 mcg/mL) is denoted as *

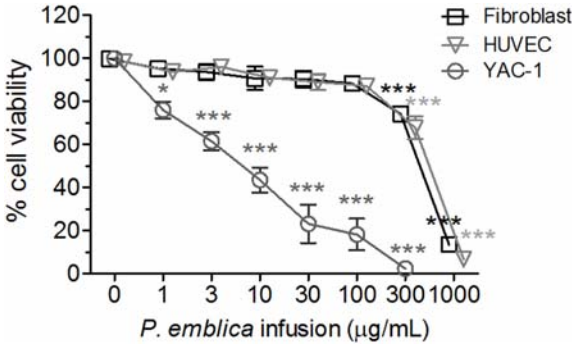

Fig. 2 The cytotoxicity of *P. emblica* infusion toward fibroblast (□, 5 x 10^3 cells/well), HUVEC (▽, 5 x 10^3 cells/well), or YAC-1 (○, 1 x 10^5 cells/well). Cells were incubated for 24 h with *P. emblica* infusion (0, 1, 3, 10, 30, 100, 300, 1,000 mcg/mL). The viability of HUVEC was measured using MTT assay. Each value represented the mean ± SEM of six independent experiments performed in triplicate (n = 18). Difference with statistical significance ($p < 0.05$, $p < 0.01$, or $p < 0.001$) from the corresponding untreated control (0 mcg/mL) is denoted as *, **, or *** respectively

For a medicinal plant, it is not feasible to identify a single compound as an active principle. The measurement of gallic acid in the studied infusion was merely employed as a fingerprint to ascertain the consistency of each preparation of infusion. Gallic acid itself might not be responsible for the observed immunomodulatory activity after the infusion ingestion.

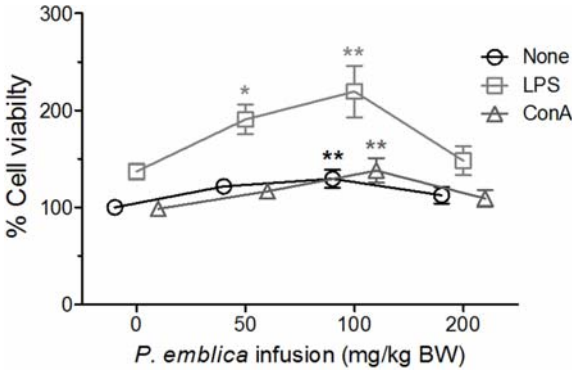

Fig. 3 Proliferative activity of isolated splenocytes from mice ingesting *P. emblica* infusion at 0, 50, 100, 200 mg/kg BW. The isolated Splenocytes were stimulated either with LPS (5 mg/mL, □), ConA (0.5 mg/mL, △), or none (○) throughout the incubation. Data were expressed as mean ± SEM of 15 mice/group. Difference with statistical significance $p < 0.05$, $p < 0.01$, or $p < 0.001$ from the splenocytes of the corresponding untreated control (0 mg/kg BW) is denoted as *, **, or *** respectively

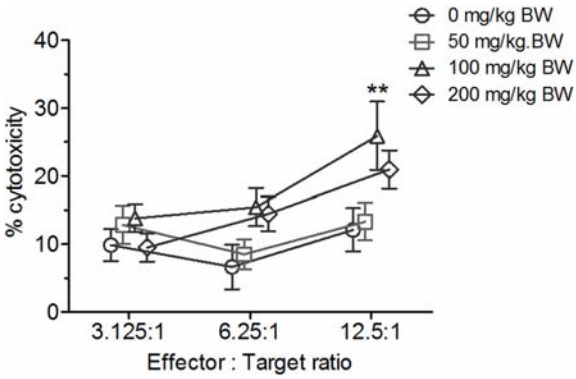

Fig. 4 NK cell derived cytotoxic activity of BALB/c mice ingesting the *P. emblica* infusion at 0 (○), 50 (□), 100 (△), or 200 (◇) mg/kg BW for 14 d (n=15/group). Each value represented the mean ± SEM. The NK cell derived cytotoxic activity was measured by the ability of splenocytes to lyse YAC-1 cells. Splenocytes were co-cultured with YAC-1 at E:T ratios of 3.125:1, 6.25:1 and 12.5:1. The cytotoxic activity was measured using propidium iodide microplate assay. Statistical significance was analyzed using two-way ANOVA with Bonferroni test. Difference with statistical significance ($p < 0.05$, $p < 0.01$, or $p < 0.001$) from the activity of splenocytes isolated from the corresponding untreated control (0 mg/kg BW) is denoted as *, **, or *** respectively

Some minor component (s) might be responsible for this activity. However, the present study did not intend to isolate the corresponding active principle, but to detect the bio-activity in animal after ingesting the whole extract of *P. emblica*.

In vitro and in vivo toxicity studies

The wide range of dose-escalating study (0, 1, 3, 10, 30, 100, 300, 1,000 mcg/mL) had been performed in normal and malignant cell lines. The employed normal cell lines included human fibroblasts and human umbilical vein endothelial cells (HUVECs), while the employed malignant cell was YAC-1 (mouse lymphoma). Using MTT colorimetric assay as a toxicity test, the IC50 (inhibitory concentration at 50th percentile) for fibroblast and HUVEC (> 300 mcg/mL) were comparable, but much higher than that of YAC-1 (< 10 mcg/mL). YAC-1 has far greater sensitivity to *P. emblica* infusion than both normal cell types (*i.e.*, fibroblast and HUVEC). The calculated therapeutic index (IC50 of fibroblast or HUVEC divided by IC50 of YAC-1) from these *in vitro* anticancer assays is greater than 30. This could be translated as a wider safety concentration range for the normal cells than those available for malignant cells. The difference in the susceptibility to *P. emblica* infusion might implicate the application of *P. emblica* infusion in the treatment of malignant conditions. However, the oral administration of *P. emblica* infusion might result in lessening toxicity as well as lessening the concentration of the active ingredient(s) due to the first pass effect. The oral route of administration might not achieve the plasma concentration required for eliminating malignant cells while sparing other normal cells. Intravenous administration or intra-tumoral administration might be required to achieve any therapeutic effect. A similar study in mouse splenocytes not only provided no evidence of toxicity, but enhanced the proliferation of the splenocytes. This *in vitro* study suggests that *P. emblica* infusion has immunostimulatory effect. The immunological effect of *P. emblica* has earlier been reported[7,9,18]. *P. emblica* alleviated the immunosuppressive effects of chromium on lymphocyte proliferation and restored the production of IL-2 and IFN-γ[18].

The anti-carcinogenic activity of the extracts with normal cell sparing has been reported. The extract had high cytotoxic effect on human lung cancer cell lines and less effect on human bronchial epithelium cell line[22]. The underlying mechanism by which *P. emblica* inhibits cancer cells is still not clear. Several possible mechanisms have been proposed involving

an interference with the cell cycle[9]. The extract showed a cell-cycle specific inhibition by inhibiting cdc25 phosphatase and cdc 2 kinase[9].

Based on the result of the *in vitro* studies, the *in vivo* experiments were conducted to determine immunostimulating effects occurring in BALB/c mice. The toxicity was evaluated after 14-day administration. The study suggested no toxicity of the *P. emblica* infusion in BALB/c mice at doses ranging from 50-200 mg/kg body weight. Therefore, the toxicity studies indicate that *P. emblica* infusion is relatively safe and is in agreement with the study for acute and chronic toxicity of the water extract of *P. emblica* fruit administered as a single oral dose of 5,000 mg/kg to rats[14]. There was no evidence of toxicity as monitored by general behavior change, mortality, or change in gross appearance of internal organs (LD50 > 5,000 mg/kg)[14].

Immunostimulatory effects

The authors determined the effect of *P. emblica* infusion on the proliferative response of lymphocytes after the stimulation with one of the two different mitogens, ConA and LPS. Splenocytes from mice have been used for the study of immune-modulating activity of herbal recipes. The splenocyte proliferation induced by B cell mitogen, LPS was significantly enhanced *P. emblica* infusion at 50 and 100 mg/kg BW, but T lymphocyte proliferation induced by ConA was significantly enhanced by *P. emblica* infusion only at the dose 100 mg/kg BW. It is concluded that *P. emblica* infusion could increase the proliferation response of splenocytes in a dose-response relationship. The present study also suggested that ingesting *P. emblica* infusion at the studied doses is safe for immune cells *in vivo* as 100% viability of freshly-isolated splenocytes was achieved. The present study provides the experimental evidence that *P. emblica* infusion has beneficial effects on immune cells, which is highly desirable in anti-cancer therapy.

Concerning the effect of *P. emblica* toward innate immune response, NK cell-mediated cytotoxic assay was performed using splenocytes isolated from mice ingesting *P. emblica* as effector cells and YAC-1 as target cells. The *P. emblica* infusion had been expected to yield a promotion of NK cell activity in earlier report. Only mice ingesting 100 mg/kg BW *P. emblica* infusion exhibited enhancement of NK activity at the E:T ratio of 12.5:1. According to the overall trend, it remains possible that higher E:T ratios would achieve significant activity over the control group. However, other investigators utilized comparable range of E:T ratios and observed clear enhancement in NK cell activity[23]. The relatively lower enhancement of NK cell activity in most groups might be attributed to the exposure of *P. emblica* infusion to heat during preparation which might have degraded some heat-labile constituents[24]. It is possible that the infusion preparation discarded some lipophilic component(s) that were essential for promoting this activity. This finding, however, substantiated the notion that *P. emblica* promoted NK cell activity.

Future directions

Based on the immunological activity of *P. emblica* infusion, the authors have alluded to the possible stronger immunological activity in an alternative preparation that retains more hydrophobic constituents. Whether or not the current or the aforementioned hydrophobic preparation elicits stronger immunological activity, the data already warrant the investigation of immunological activity in HPLC fractions of *P. emblica*. Further fractionation of the extract would eventually lead to the discovery of the active principle of *P. emblica*.

Conclusion

The present study of *P. emblica* focused on 2 immunological efficacy models, *in vitro* and *in vivo*. The *in vitro* study incorporated the direct cytotoxicity of *P. emblica* infusion toward normal cells (mouse splenocytes, human fibroblast, HUVECs) and cancer cells (YAC-1). *P. emblica* was relatively safer for normal cells than the cancer cells. *P. emblica* infusion below toxicity dose has immunostimulatory effects by enhancing the proliferation of splenocyte from BALB/c mice. The infusion strongly augmented the lymphoproliferative response to the B cell mitogen, LPS; as well as to the T cell mitogen, ConA. The *in vivo* study incorporated the immunostimulatory activities in BALB/c mice ingesting *P. emblica* infusion. Mice ingesting *P. emblica* infusion for 14 d carried healthy splenocytes with higher proliferative potential in a dose-dependent relationship. The increase in proliferative activity was in good agreement with the *in vitro* study. There was some enhancement in NK cell activity in the isolated splenocytes from mice ingesting 100 mg/kg BW *P. emblica* infusion. It is concluded that *P. emblica* infusion was relatively benign to normal cells. The infusion contained growth enhancing activity toward mice splenocytes as well as enhancing NK cell activity. The overall study strengthens our belief that *P. emblica*

infusion is a useful immunomostimulant with a potential as an adjuvant for anti-cancer agents.

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Potential conflicts of interest

None.

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ผลของชาชงมะขามป้อมต่อการกระตุ้นภูมิคุ้มกันของหนู

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ภูมิหลัง: *มะขามป้อม (Phylllanthus emblica L.) ถูกใช้เป็นส่วนประกอบของตำรับยาแพทย์แผนไทย เพื่อชะลอความชราหรืออายุวัฒนะ หนึ่งในกลไกสำคัญของการชะลอความชราคือการกระตุ้นระบบภูมิคุ้มกัน*

วัตถุประสงค์: *1) เพื่อสำรวจประสิทธิผลของชาชงมะขามป้อมต่อระบบภูมิคุ้มกัน ซึ่งเป็นส่วนหนึ่งของการชะลอความชรา ในหนูทดลองชนิด BALB/c 2) เพื่อตรวจสอบความปลอดภัยของการบริโภคชาชงมะขามป้อมในหนูทดลอง*

วัสดุและวิธีการ: *การศึกษาฤทธิ์ยานอกร่างกาย ทำโดยการวัดความสามารถแบ่งตัวของเซลล์ชนิดต่างๆ คือเซลล์เม็ดเลือดขาวที่เตรียมจากม้ามหนู เซลล์เยื่อบุผนังหลอดเลือด เซลล์ fibroblasts และเซลล์มะเร็งเม็ดเลือดขาว (YAC-1) ที่สัมผัสกับชาชงมะขามป้อมโดยตรง การศึกษาในสัตว์ทดลอง ทำโดยให้หนูกลืนชาชงมะขามป้อมในขนาด 0, 50, 100 หรือ 200 มิลลิกรัม/น้ำหนักตัว 1 กิโลกรัม เป็นเวลา 14 วัน แล้วเตรียมเซลล์เม็ดเลือดขาวจากม้ามของหน ทุกกลุ่มมาทดสอบการแบ่งตัวของเซลล์และฤทธิ์การทำลายเซลล์มะเร็งเม็ดเลือดขาว*

ผลการศึกษา: *การศึกษาในหลอดทดลอง ชาชงมะขามป้อมสามารถเพิ่มการแบ่งตัวโดยตรงของเซลล์เม็ดเลือดขาวที่เตรียมจากม้ามหนูซึ่งการแบ่งตัวนี้เพิ่มขึ้นตามความเข้มข้นของชาชงมะขามป้อม นอกจากนี้ขนาดของชาชงมะขามป้อมที่ใช้ในการศึกษาแสดงความเป็นพิษต่อเซลล์มะเร็งเม็ดเลือดขาว YAC-1 ของหนูในขณะที่ไม่พบความเป็นพิษต่อเซลล์ปกติทั้งเซลล์เยื่อบุผนังหลอดเลือด เซลล์ fibroblasts*

การศึกษาในสัตว์ทดลองพบว่า เซลล์เม็ดเลือดขาวที่เตรียมจากม้ามของหนูกลุ่มที่ได้รับชาชงมะขามป้อมมีการแบ่งตัวเพิ่มขึ้นตามขนาดของชาชงมะขามป้อมที่ได้กลืน ส่วนการทดสอบฤทธิ์ทำลายเซลล์มะเร็งเม็ดเลือดขาวโดย NK cell ของหนูนั้นมีเพียงหนูที่ได้รับชาชงมะขามป้อมขนาด 100 มิลลิกรัม/น้ำหนักตัว 1 กิโลกรัม ที่สามารถทำลายเซลล์มะเร็งเพิ่มขึ้น

สรุป: *ชาชงมะขามป้อมสามารถเร่งการแบ่งตัวของเซลล์เม็ดเลือดขาวที่เตรียมจากม้ามหนูทั้งในกรณีที่สัมผัสกับชาชงมะขามป้อมโดยตรง กับทั้งในหนูที่กลืนชาชงมะขามป้อม แต่ฤทธิ์เร่งความสามารถของ NK cell ในการทำลายเซลล์มะเร็งมีจำกัด ขนาดของชาชงมะขามป้อมที่ใช้มีความปลอดภัยตลอดการศึกษา*