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A study on the effects of herbal acupuncture with *Liriopis Tuber* extract on airway inflammation in the mouse induced with bronchial asthma

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ABSTRACT

Objectives: Herbal acupuncture has been administered with *Liriopis Tuber* extract on the point of BL 13 (*Pyesu*) to treat bronchial asthma and a certain degree of clinical benefits have been observed but lacking scientific substantiation.

Methods: The present report describes on Th1 cytokine (Interleukin-2, Interferon -gamma), Th2 cytokine, (Interleukin-4, Interleukin-5), and IL-12 in bronchoalveolar lavage fluid (ELISA).

Five groups were devised to study the effects of herbal-acupuncture with *Liriopis Tuber* extract at BL 13 (*Pyesu*) for airway inflammation in the mouse model with bronchial asthma.

Results shows that herbal acupuncture with *Liriopis Tuber* extract at BL 13 increased Th1 cytokine (Interleukin-2) in allergic sensitization and allergic challenge, and decreased Th2 cytokine (Interleukin-4, Interleukin-5) in allergic sensitization.

Key word: BL 13(*Pyesu*), *Liriopis Tuber* extract, cytokine, allergy, herbal acupuncture

I . Introduction

Allergic asthma in Oriental medicine is caused by coldness, psychological factors, genetic causes, phlegm, oversensitivity, and decline in the functions of visceral organs such as lung, spleen, kidneys, and etc. Accompanying symptoms are wheezing, cough, dyspnea, and other bronchial signs.

Herbal acupuncture is a treatment method that injects various types of herbal extract on the acupuncture points, area of positive reaction, and pressure points. This is a new method[6-7] that combines benefits of both acupuncture and herbal medicine to control the bodily functions and improve the physiological state.

Liriopis Tuber is an herb in the lily family that is collected in the summer time and dried. It enters lung, stomach, and heart meridians and has characteristics of lubricating the lung, pacify, benefit the stomach and generating fluid, thus effective in treating lung dryness, hemoptysis, lung hemorrhage, and dry mouth[8-10].

BL 13 is located 1.5 cun lateral to the mid-vertebral line, at the level of the lower border of the spinous process of the third thoracic vertebra. BL 13 is popularly used for the diseases associated with lung disorders[11-12].

Allergic bronchial asthma is closely associated with the reaction of IgE that is controlled by either T lymphocyte and B lymphocyte, and activated by mutual operation between the antigen and adipose cell-IgE molecule[13]

This experiment was conducted to examine the immunity and anti-allergic effects of *Liriopis Tuber* herbal acupuncture in BALB/c mouse with allergic asthma induced by ovalbumin. Then *Liriopis Tuber* herbal extract was injected on BL 13 in relation to the time of antigen reduction and induction, and

compared the effects on manifestation of T-lymphocyte cytokine interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-12 (IL-12), and interferon-gamma (IFN-γ).

Ⅱ. Experiment Materials and Methods

1. Animals and materials

1) Experiment animals

Animals used in the experiment were 6 weeks old female balb/c mice weighing approximately 20-25 grams, supplied by Korea Research Institute of Bioscience & Biotechnology - Laboratory Animal Division. The animals were given solid pellet feed (Samfeed Inc., Korea) free of antibiotics and plenty of water, and were adapted to the laboratory for 7-10 days before conducting the experiment at the room temperature (22±2℃). Eight mice constitute as one experiment group.

2) Materials and Methods

Liriopis Tuber used in this experiment was purchased by Sangji University affiliated hospital and carefully selected. Water used for extraction was distilled with an ion exchange resin for three times.

2. Preparation of *Liriopis Tuber* herbal extract

1)*Liriopis Tuber*[8-10] herbal acupuncture extract

300g of *Liriopis Tuber* was inserted to a round flask (reflux cooler attached) with 1000ml of distilled water and decocted for 3 hours. Then the decoction was decompressed using a rotary evaporator (Yamato, Japan) until the total volume reached 200ml and cooled to a room temperature. Then 200ml of 95% ethanol was added and the formed precipitate was filtered.

Remaining fluid was again decompressed with the rotary evaporator and the formed precipitate was filtered. Then 200ml of 95% ethanol was added and the formed precipitate was filtered. Remaining fluid was once again decompressed with the rotary evaporator and added 200ml of 75% ethanol and the proceduure was repeated twice. Then remaining fluid was adjusted to 200ml by pressurizing the ethanol and the pH balance was adjusted to 7.2-7.4 with 0.1N of NaOH. Vacuum filtrator (Millipore, USA) with membrane filter

(0.45㎛, 25mm diameter) was pressure sterilized before filtering and the solution was filtered again with syringe filter (0.25㎛, Whatman, U.S.A) and pressure sterilized again before used as an herbal acupuncture extract.

2) Herbal extract injector

30 gauge 1ml insulin syringe (Becton Dickinson, U.S.A) was soaked in 70% ethanol for 12 hours before the usage.

3. Experiment Methods[17)]

1) Acupuncture point and method

Point at the level of the lower border of the spinous process of the third thoracic vertebra, 0.5cm bilateral points that correspond to the human BL 13[11-12)] were injected with 01.cc of the herbal extract once a day alternating the side.

2) Bronchial asthma model

Ovalbumin was injected in either abdominal cavity or hypodermis of the mouse to sensitize and after 2-4 weeks, ovalbumin was injected in the airway to induce allergic asthma. When the asthma was induced, the mouse experiences contraction in the trachea and becomes overly sensitive, and eosinophils and Th2 cytokine build up in the trachea which mimics human allergic asthma.

3) Experiment - day 1 (sensitization - day 1)

10% aluminum sulfate (Sigma, St. Louis, MO) 1㎖ was mixed with 500㎍ of ovalbumin (Pierce, Rockford, IL) and the pH was balanced to 6.5. After 30-50 minutes at the room temperature, the solution was centrifuged for 5 minutes at 750×g. Then the saline solution was added to the precipitate to make 1㎖, and 100㎕ was injected to the abdominal cavity.

4) Experiment - day 15 (re-sensitization)

Above ovalbumin was injected in the abdominal cavity and nasal cavity to re-sensitize. For sensitization in the nasal cavity, 10% aluminum sulfate (Sigma, St. Louis, MO) 1㎖ mixed with 2000㎍ of ovalbumin (Pierce, Rockford, IL) and injected 50㎕. Injection in the nasal cavity was done under anesthesia, and anesthetic agents of ketamin (0.44㎎/㎖) and xylazin (6.3㎎/㎖) 0.25㎖ were injected in the abdominal cavity.

5) Experiment - day 26,27,28

Above ovalbumin was injected in the

nasal cavity every day and induced asthma. 10% aluminum sulfate (Sigma, St. Louis, MO) 1㎖ mixed with 2000㎍ of ovalbumin (Pierce, Rockford, IL) and injected 50㎕ to the nasal cavity. Injection in the nasal cavity was done under anesthesia, and ketamin (0.44㎎/㎖) and xylazin (6.3㎎/㎖) 0.25㎖ were used as anesthetic agents.

6) Classification of experiment groups

Treament (uisng *Liriopis Tuber* herbal extract)

◇ Group 1 - normal : 8 Balb/c mice without any treatment

◇ Group 2 - control : Induced allergic bronchial asthma with ovalbumin, but untreated.

◇ Group 3 - sensitized : Induced allergic bronchial asthma with ovalubumin, and treated with the Liriopis Tuber herbal extract from day 1 to day 5 on the alternating point of BL 13 once a day.

◇ Group 4 - induced : Induced allergic bronchial asthma with ovalubumin, and treated with the Liriopis Tuber herbal extract from day 24 to day 28 on the alternating point of BL 13 once a day.

◇ Group 5 - sensitized and induced : Induced allergic bronchial asthma with ovalubumin, and treated with the Liriopis Tuber herbal extract from day 1 to day 5, and day 24 to day 28 on the alternating point of BL 13 once a day.

4. Cleaning of bronchial germinal cells

After conclusion of experiment procedures, the object was anesthetized with urethane and dissected. Lung and bronchus were removed and placed in 10ml of the saline solution, then pulverized with homogeniger (Heidolph, Germany), followed by centrifuge at 4℃, 300×g for 3 times. Upper portion solution was stored at -20℃.

5. Production and measurement of cytokine[17)]

Lung and bronchus were removed and placed in 10ml of the saline solution, then pulverized with homogeniger (Heidolph, Germany), followed by centrifuge at 4℃, 300×g for 3 times. Upper solution (bronchoalveolar lavage fluid) was stored at -20℃ and measured IL-2, IL-4, 1L-5, IL-12, and IFN-γ.

6. Statistical evaluation[18,19)]

Authorization of significance was decided by student's t-test and p-value

must be at least 0.05 to be determined as significant. Using SAS and analytical ratio, each group's average was determined for significancy and compared with Duncan's multiple-ranger test at α =0.05.

Ⅲ. Results

1. Effects on the Interleukin-2 productivity

Normal group's productivity was measured at 226.7±63.1pg/ml, control group at 156.6±33.9pg/ml, group 3 at 220.2±71.6pg/ml, group 4 at 271.5±123.7pg/ml, and group 5 resulted at 228.9±35.9pg/ml, thus all the groups showed increase but only the group 5's productivity was labeled with significant increase ($p<0.05$).

For individual comparison using the Duncan's multiple-range test, the group 4 showed significant increase over the control group , and the group 3 and 5 didn't show significant changes. Difference between the group 4, 3, and 5 was insignificant. (Table Ⅰ, Fig. 1).

2. Effects on the Interleukin-4 productivity

Normal group's productivity was measured at 92.2±48.6pg/ml, control group at 188.7±60.5pg/ml, group 3 at 47.6±12.0pg/ml, group 4 at 158.5±46.7pg/ml, and group 5 resulted at 134.2±96.6pg/ml, thus group 3 showed

Table Ⅰ. IL-2 Production potential level in bronchoalveolar lavage fluid.

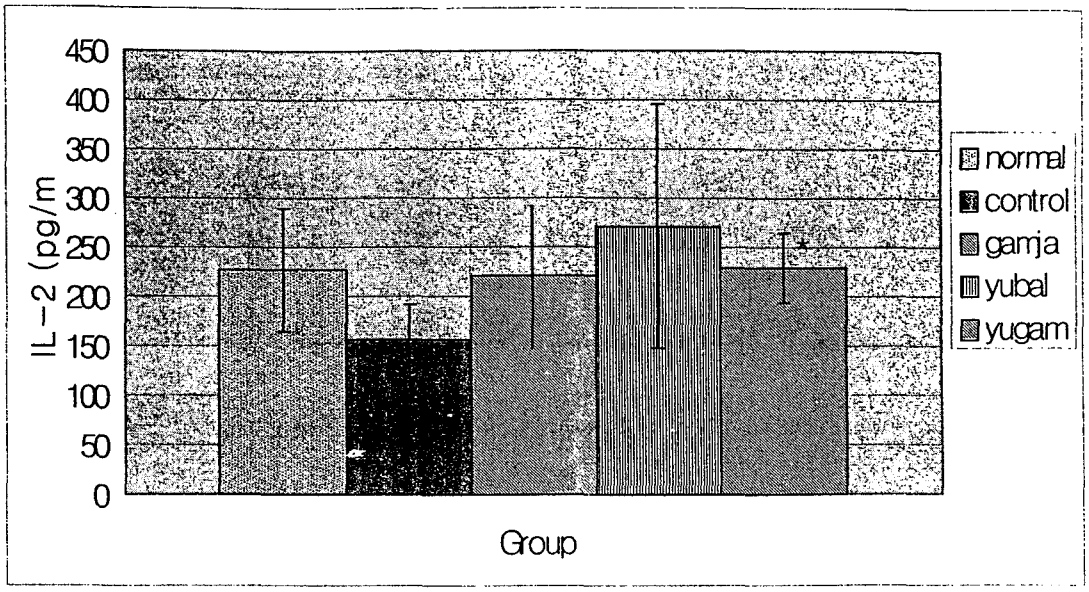
Group	No. of animals	IL-2 Production potential (pg/ml)	Duncan Grouping
Normal	8	226.7±63.1[a)]	AB[b)]
Control	8	156.6±33.9	A
3	8	220.2±71.6	AB
4	8	271.5±123.7	B
5	8	228.9±35.9*	AB

a) : Mean±Standard error..

b) : Means with different letters (A,B) within a column are significantly different from each other α=0.05 as determined by Duncan's multiple-range test.

* : Statistical significance compared with control data(*: $p<0.05$).

Fig. 1. IL-2 Production potential level in bronchoalveolar lavage fluid.



Normal: Non treated group.

Control : Induced allergic bronchial asthma with ovalbumin, but untreated.

3 - sensitized : Induced allergic bronchial asthma with ovalubumin, and treated with the *Liriopis Tuber* herbal extract from day 1 to day 5 on the alternating point of BL 13 once a day.

4 - induced : Induced allergic bronchial asthma with ovalubumin, and treated with the *Liriopis Tuber* herbal extract from day 24 to day 28 on the alternating point of BL 13 once a day.

5 - sensitized and induced : Induced allergic bronchial asthma with ovalubumin, and treated with the *Liriopis Tuber* herbal extract from day 1 to day 5, and day 24 to day 28 on the alternating point of BL 13 once a day.

* : Statistical significance compared with control data(*: $p<0.05$).

significant decrease ($p<0.05$) compared to the control group. Group 4 and 5 figured at reduced productivity, but insignificant.

For individual comparison using the Duncan's multiple-range test, the group 3 showed significant increase over the control group, and the group 3 and 5 didn't show significant changes. Significant difference was found between the group 3 and 4, 5. (Table II, Fig. 2).

3. Effects on the Interleukin-5 productivity

Normal group's productivity was measured at 3.2±0.4pg/ml, control group at 3.9±0.1pg/ml, group 3 at 3.3±0.1 pg/ml, group 4 at 5.1±2.1pg/ml, and

Table II. IL-4 Production potential level in bronchoalveolar lavage fluid.

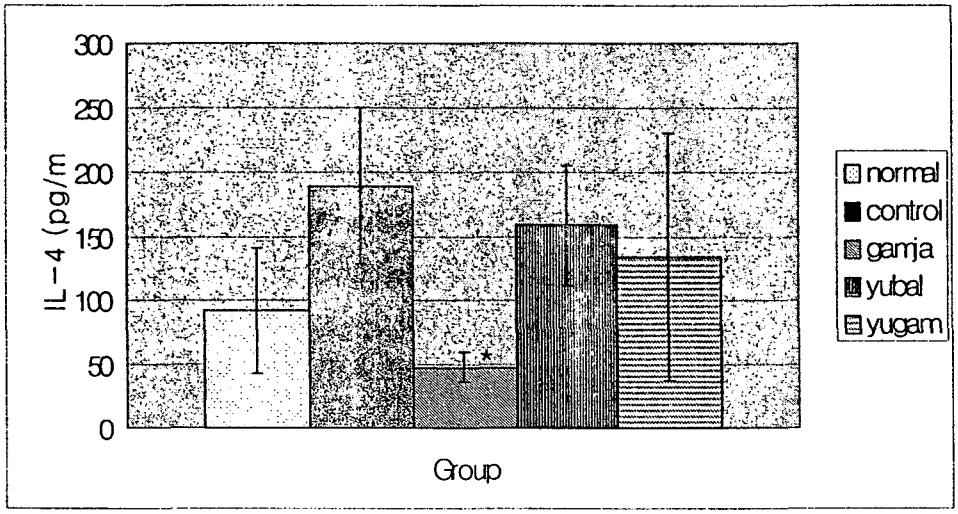
Group	No of animals	IL-4 Production potential (pg/ml)	Duncan Grouping
Normal Group	8	92.2±48.6[a)]	AC[b)]
Control Group	8	188.7±60.5	B
3	8	47.6±12.0*	A
4	8	158.5±46.7	BC
5	8	134.2±96.6	BC

a) : Mean±Standard error.

b) : Means with different letters(A,B) within a column are significantly different from each other α=0.05 as determined by Duncan's multiple-range test.

* : Statistical significance compared with control data(*: $p<0.05$).

Fig. 2. IL-4 Production potential level in bronchoalveoar lavage fluid.



Normal: Non treated group.

Control : Induced allergic bronchial asthma with ovalbumin, but untreated.

3 - sensitized : Induced allergic bronchial asthma with ovalubumin, and treated with the *Liriopis Tuber* herbal extract from day 1 to day 5 on the alternating point of BL 13 once a day.

4 - induced : Induced allergic bronchial asthma with ovalubumin, and treated with the *Liriopis Tuber* herbal extract from day 24 to day 28 on the alternating point of BL 13 once a day.

5 - sensitized and induced : Induced allergic bronchial asthma with ovalubumin, and treated with the *Liriopis Tuber* herbal extract from day 1 to day 5, and day 24 to day 28 on the alternating point of BL 13 once a day.

* : Statistical significance compared with control data(*: $p<0.05$).

group 5 resulted at 4.2±0.4pg/ml, thus the control group showed significant increase compared to the normal group. Group 3 displayed significant decrease ($p<0.05$) over the control group. Group 4 and 5 showed increase compared to the control group, but insignificant.

For individual comparison using the Duncan's multiple-range test, the groups of 3, 4, and 5 failed to show signigicant differences compared to the control group. However, significant difference was acknowledged between the group 4 and 5. (Table Ⅲ, Fig. 3).

4. Effects on the Interleukin-12 productivity

Normal group's productivity was measured at 203.4±16.1pg/ml, control group at 194.3±39.1pg/ml, group 3 at 338.1±279.2pg/ml, group 4 at 225.0± 18.2pg/ml, and group 5 resulted at 190.6 ±30.0pg/ml, thus the control group showed insignificant decrease over the normal group. Group 4 and 5 showed increase over the control group, but the value was insignificant.

For individual comparison using the Duncan's multiple-range test, the groups of 3, 4, and 5 failed to show signigicant differences compared to the control group. (Table Ⅳ, Fig. 4).

5. Effects on the Interferon-γ productivity

Normal group's productivity was measured at 2515.0±449.8pg/ml, control group at 1888.2±276.4pg/ml, group 3 at 2373.0±788.9pg/ml, group 4 at 1623.4± 170.2pg/ml, and group 5 resulted at 1699.8±214.4pg/ml, thus the control group showed insignificant decrease over the normal group.

Group 3 showed increase compared to the control group, but the difference is insignificant. On the other hand, group 4 and 5 displayed decrease over the control group, yet again, the difference is insignificant.

For individual comparison using the Duncan's multiple-range test, the groups 3 was acknowledged with significant increase over the control group. Group 4 and 5 failed to display significant increase compared to the control group. Between the groups of 3, 4, and 5, significant difference was demonstrated. (Table Ⅴ, Fig. 5).

Table III. IL-5 Production potential level in bronchoalveolar lavage fluid.

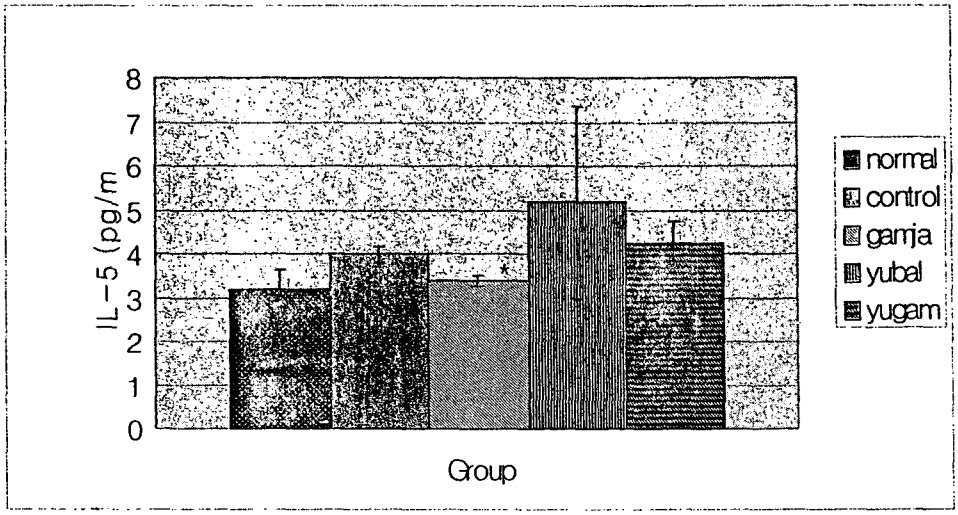
Group	No of animals	IL-5 Production potential (pg/ml)	Duncan Grouping
Normal Group	8	3.2±0.4[a)]	A[b)]
Control Group	8	3.9±0.1	AB
Gamjak	8	3.3±0.1*	A
Yubal	8	5.1±2.1	B
Yu-gam	8	4.2±0.4	AB

a) : Mean±Standard error.

b) : Means with different letters(A,B) within a column are significantly different from each other α=0.05 as determined by Duncan's multiple-range test.

* : Statistical significance compared with control data(*: $p<0.05$).

Fig. 3. IL-5 Production potential level in bronchoalveolar lavage fluid.



Normal: Non treated group.

Control : Induced allergic bronchial asthma with ovalbumin, but untreated.

3 - sensitized : Induced allergic bronchial asthma with ovalubumin, and treated with the *Liriopis Tuber* herbal extract from day 1 to day 5 on the alternating point of BL 13 once a day.

4 - induced : Induced allergic bronchial asthma with ovalubumin, and treated with the *Liriopis Tuber* herbal extract from day 24 to day 28 on the alternating point of BL 13 once a day.

5 - sensitized and induced : Induced allergic bronchial asthma with ovalubumin, and treated with the *Liriopis Tuber* herbal extract from day 1 to day 5, and day 24 to day 28 on the alternating point of BL 13 once a day.

* : Statistical significance compared with control data (*: $p<0.05$).

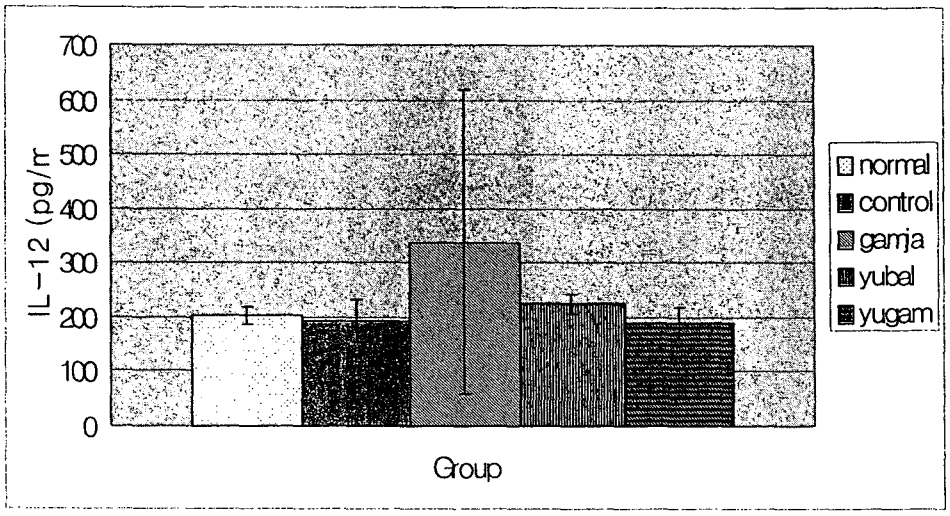
Table Ⅳ. IL-12 Production potential level in bronchoalveolar lavage fluid.

Group	No of animals	IL-12 Production potential (pg/ml)	Duncan Grouping
Normal Group	8	203.4±16.1[a)]	A[b)]
Control Group	8	194.3±39.1	A
Gamjak	8	338.1±279.2	A
Yubal	8	225.0±18.2	A
Yu-gam	8	190.6±30.0	A

a) : Mean±Standard error.

b) :Means with different letters(A,B) within a column are significantly different from each other α=0.05 as determined by Duncan's multiple-range test.

Fig. 4. IL-12 Production potential level in bronchoalveolar lavage fluid.



Normal: Non treated group.

Control : Induced allergic bronchial asthma with ovalbumin, but untreated.

3 - sensitized : Induced allergic bronchial asthma with ovalubumin, and treated with the *Liriopis Tuber* herbal extract from day 1 to day 5 on the alternating point of BL 13 once a day.

4 - induced : Induced allergic bronchial asthma with ovalubumin, and treated with the *Liriopis Tuber* herbal extract from day 24 to day 28 on the alternating point of BL 13 once a day.

5 - sensitized and induced : Induced allergic bronchial asthma with ovalubumin, and treated with the *Liriopis Tuber* herbal extract from day 1 to day 5, and day 24 to day 28 on the alternating point of BL 13 once a day.

* : Statistical significance compared with control data (*: $p<0.05$).

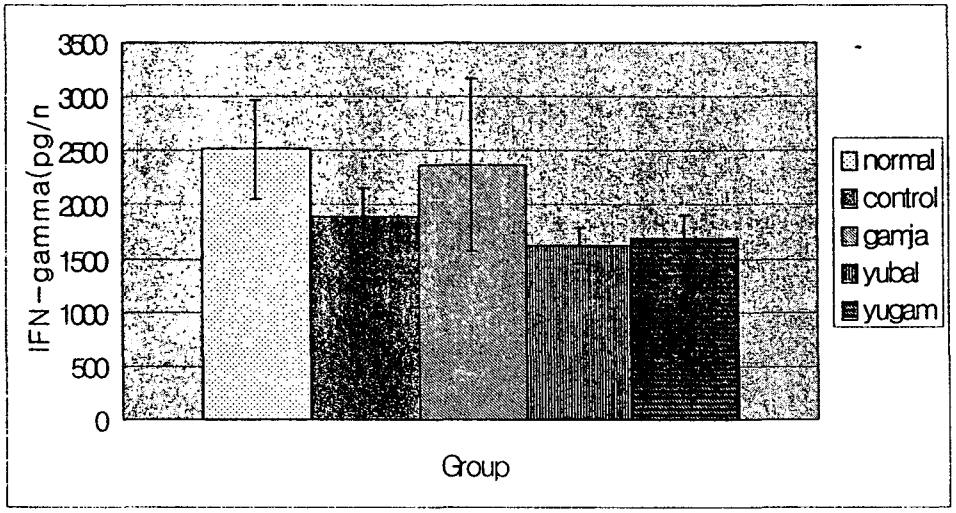
Table V. IFN-γ Production potential level in bronchoalveolar lavage fluid.

Group	No of animals	IFN-γ Production potential (pg/ml)	Duncan Grouping
Normal Group	8	2515±449.8[a)]	A[b)]
Control Group	8	1888.2±276.4	BC
Gamjak	8	2373±788.9	A
Yubal	8	1623.4±170.2	C
Yu-gam	8	1699.8±214.4	C

a) : Mean±Standard error.

b) : Means with different letters(A,B) within a column are significantly different from each other α=0.05 as determined by Duncan's multiple-range test.

Fig.5. IFN-γ Production potential level in bronchoalveolar lavage fluid.



Normal: Non treated group.

Control : Induced allergic bronchial asthma with ovalbumin, but untreated.

3 - sensitized : Induced allergic bronchial asthma with ovalubumin, and treated with the *Liriopis Tuber* herbal extract from day 1 to day 5 on the alternating point of BL 13 once a day.

4 - induced : Induced allergic bronchial asthma with ovalubumin, and treated with the *Liriopis Tuber* herbal extract from day 24 to day 28 on the alternating point of BL 13 once a day.

5 - sensitized and induced : Induced allergic bronchial asthma with ovalubumin, and treated with the *Liriopis Tuber* herbal extract from day 1 to day 5, and day 24 to day 28 on the alternating point of BL 13 once a day.

* : Statistical significance compared with control data (*: $p<0.05$).

Ⅳ. Discussion

Herbal acupuncture therapy is also known as an acupuncture point injection therapy that combines efficacies of both acupuncture therapy and herbal medication. Herbal extract is injected directly on the acupuncture points based on the meridian theory or on the point of sore pressure. The benefits of the herbal acupuncture include quicker absorption of the medication and can be applied when oral administration is impossible[7]. Other possible effects of the herbal acupuncture are analgesic, detoxification, tonify blood, decrease in blood pressure, anti-tumor, anti-inflammation, among others[22,23]. In this experiment, we've chosen leading anti-cough and yin tonic herb of *Liriopis Tuber* and injected on the point of BL 13 that governs the functions of lung[11-12].

Allergic asthma in Oriental medicine is caused by coldness, psychological factors, genetic causes, phlegm, oversensitivity, and decline in the functions of visceral organs such as lung, spleen, kidneys, and etc. Accompanying symptoms are wheezing, cough, dyspnea, and other bronchial signs.

Allergic bronchial asthma is a leading type of allergic disorder that is characterized by oversensitive reaction to the deposit of eosinophils, adipose cells, T-lymphocytes and other activated inflammatory cells on the airway[17]. Activated eosinophils release granulate proteins and arachidonate metabolic substances and cause constriction of the smooth muscles in the bronchus and falling out of epithelial cells. And cytokine excreted by T-helper (Th) cell plays vital role in the activation of the eosinophils. Depending on the cytokine excretion pattern, T-helper cell is classified into either Th1 that excretes IL-2 and IFN-γ, whereas Th2 excretes IL-4, IL-5, IL-10, and IL-13. For allergic bronchial asthma, Th2 cytokine plays important role in the migration and activation of the eosinophils[17,22,23].

Ovalbumin is injected in either abdominal cavity or hypodermis of the mouse to sensitize and after 2-4 weeks, ovalbumin is injected in the airway to induce allergic asthma. When the asthma is induced, the mouse experiences contraction in the trachea and becomes overly sensitive, and eosinophils and Th2 cytokine build up in the airway which

mimics human allergic asthma. Sensitization period is referred to the time when Th0 cell differentiates into Th2, and induction period is when Th0 cell finishes the differentiation into Th2 cell[17].

Interleukin-2 (IL-2) is a T cell growth factor that was discovered by Mogan. It's a type of lymphokine that is made by helper T cell and LGL (large granular lymphocyte) which are stimulated by antigen specific or non-specific. IL-2 is concerned with the increase or decrease of the immune system with production of natural killer cells, phagocytes, and cytotoxic lymphocytes [23,24].

By examining the effects on the IL-2 productivity, normal group's productivity was measured at 226.7±63.1pg/ml, control group at 156.6±33.9pg/ml, group 3 at 220.2±71.6pg/ml, group 4 at 271.5± 123.7pg/ml, and group 5 resulted at 228.9 ±35.9pg/ml, thus all the groups showed increase but only group 5's productivity was labeled with significant increase ($p<0.05$). (Table I , Fig. 1)

As other types of interleukin, IL-4 also effects various types of cells and has diverse functions. It works as the growth factor for the T cells and adipose cells and it's known to be the only cytokine that plays part in the IgE antibody[25]. By examining the effects on the IL-4 productivity, normal group's productivity was measured at 92.2±48.6pg/ml, control group at 188.7±60.5pg/ml, group 3 at 47.6±12.0pg/ml, group 4 at 158.5± 46.7pg/ml, and group 5 resulted at 134.2 ±96.6pg/ml, thus group 3 showed significant decrease ($p<0.05$) compared to the control group. Group 4 and 5 figured at reduced productivity, but insignificant. (Table II, Fig. 2)

Interleukin 5 (IL-5) is a cytokine that proliferates and differentiates the B cells just as Il-4, and accelerates the differentiation rate of the B cells with IL-2 or IL-4. It also plays part in the IgA production and eosinophil mediated inflammation[22,23]. By examining the effects on the IL-5 productivity, normal group's productivity was measured at 3.2 ±0.4pg/ml, control group at 3.9± 0.1pg/ml, group 3 at 3.3±0.1pg/ml, group 4 at 5.1±2.1pg/ml, and group 5 resulted at 4.2±0.4pg/ml, thus the control group showed significant increase compared to the normal group. Group 3 displayed significant decrease ($p<0.05$) over the

control group. Group 4 and 5 showed increase compared to the control group, but insignificant. (Table Ⅲ , Fig. 3)

Interluekin-12 (IL-12) differentiates into Th1 in the initiation stage of the immune response. Szabo[26] and others reported that Th1 cell can be transformed into Th2 if stimulated by IL-4, and Th2 cell can be transformed into Th1 if stimulated by IL-12[27]. By examining the effects on the IL-12 productivity, normal group's productivity was measured at 203.4±16.1pg/ml, control group at 194.3±39.1pg/ml, group 3 at 338.1±279.2pg/ml, group 4 at 225.0 ±18.2pg/ml, and group 5 resulted at 190.6±30.0pg/ml, thus the control group showed insignificant decrease over the normal group. Group 4 and 5 showed increase over the control group, but the value was insignificant. (Table Ⅳ , Fig. 4)

IFN-$\gamma$is known to suppress the IgE production by IL-4[22,23]. By examining the effects on the IFN-γ productivity, normal group's productivity was measured at 2515.0±449.8pg/ml, control group at 1888.2±276.4pg/ml, group 3 at 2373.0±788.9pg/ml, group 4 at 1623.4± 170.2pg/ml, and group 5 resulted at 1699.8±214.4pg/ml, thus the control group showed insignificant decrease over the normal group. Group 3 showed increase compared to the control group, but the difference is insignificant. On the other hand, group 4 and 5 displayed decrease over the control group, yet again, the difference is insignificant. (Table Ⅴ, Fig. 5)

Integrating all the experiment data, injecting *Liriopis Tuber* herbal extraction on the BL 13 of BALB/c mouse, Th2 cytokine of Il-4 and Il-5 that promotes allergic reaction is significantly ($p<0.05$) decreased in the group 3 (sensitized). The productivity of Th1 cytokine IL-2 that suppresses allergic inflammative reaction is significantly increased ($p<0.05$) in the group 3 (sensitized and induced). Based on these results, *Liriopis Tuber* extract is more effective when given in the initiation (sensitized) stage or better yet, given in the initiation and induction stage of the allergic reaction. This also suggest that *Liriopis Tuber* herbal acupuncture can be effectively used for preventing an allergic bronchial asthma. Further researches are required to provide more data to prove specific

pathway of action and to be safely administered for the clinical usage.

V. Conclusion

This experiment was conducted to examine the immunity and anti-allergic effects of *Liriopis Tuber* herbal acupuncture in BALB/c mouse with allergic asthma induced by ovalbumin. Then *Liriopis Tuber* herbal extract was injected on BL 13 in relation to the time of antigen reduction and induction, and compared the effects on manifestation of T-lymphocyte cytokine interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-12 (IL-12), and interferon-gamma (IFN-γ).

The following results were obtained:

1. Interleukin-2 productivity was significantly increased in the group 3 (sensitized) and group 4 (induced), compared to the control group.

2. Interleukin-4 productivity was significantly decreased in only the group 3 (sensitized), compared to the control group.

3. Interleukin-5 productivity was significantly decreased in only the group 3 (sensitized), compared to the control group.

4. Interleukin-12 productivity was insignificantly changed in the experiment groups, compared to the control group.

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