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Effect of Propolis Volatiles from a Stingless Honeybee(Apidae : Meliponinae) on the Immune System of Elderly Residents in a Nursing Home

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We report an evaluation of the immunostimulatory effect of propolis volatiles from a stingless honeybee. We studied 34 elderly residents at a special nursing home. Twenty-one subjects were treated with propolis, 8 with Binchô charcoal and 5 subjects acted as controls. Subjects treated with either propolis or Binchô charcoal were housed in rooms separated from the other non-study residents in the nursing home. The effects of each treatment on natural killer (NK) cell activity and lymphocyte levels were examined after 2 months and then for a longer period. The results indicated that NK cell activity was significantly improved to that within the normal range only after propolis treatment.

Key words: Propolis, Volatile, Stingless Honeybee, Nursing home, Natural killer cell, Immune system

Introduction

Propolis, a natural resinous product that consists of buds and exudates of plants collected by honeybees plus honeybee wax, has antibacterial, antiviral, anesthetic, immunostimulatory, and anti-tumor effects (Bankova *et al*., 2000; Sforcin, 2007). Propolis is also reported to have an anti-amnesic effect on scopolamine-induced memory impairment in mice (Chen *et al*., 2008). The safe administration of propolis and its side effects on humans have been reviewed by Sforcin (2007), but there remains insufficient evidence to support its benefits to human health. Since humans have been using propolis for a long time, however, some studies claim it to make a contribution to human health.

Although propolis supplementation in the female subjects did not induce any changes in blood parameters, a decrease in the concentration of malondialdehyde and increased superoxide dismutase activity were detected in the male subjects (Jasprica *et al*., 2007). When patients with asthma were administered an aqueous extract of propolis, it proved potentially effective as an adjuvant to therapy (Khayyai *et al*., 2003). It is thus likely that scientifically-based information and evidence will be published on propolis-treated humans.

The propolis produced by European honeybee (*Apis mellifera*) (Apidae : Apinae) has been extensively studied, as described above. Bankova and Popova (2007) recently reviewed the literature on the chemical composition and biological activity of propolis from stingless honeybees (Meliponinae) in different genera; they stated that propolis from stingless honeybees demonstrated many medical properties that were both the same and different to those seen in *A. mellifera* propolis. Some samples of Meliponinae propolis have shown greater antibacterial activity than *A. mellifera* propolis (Fernandes Jr., *et al*., 2001). Although volatiles of propolis from *A. mellifera* and stingless honeybees from Yucatan were also analyzed, some monoterpenes and sesquiterpenes were found at higher levels in propolis from the stingless bee *Melipona beecheii* (Pino *et al*., 2006).

In propolis volatiles, a number of new components were identified, chiefly mono- and sesqui-terpenoids (Bankova *et al*., 2000), but there are no previous studies on the beneficial effects of propolis volatiles on the health of elderly people. In the present paper, we describe the pharmacological effects of propolis volatiles from the stingless honeybees *M. beecheii* on the immune systems of elderly residents of a nursing home.

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Materials and Methods

Preparation of stingless honeybee propolis

Propolis produced by the stingless honeybee *Melipona beecheii* was kindly provided by Professor M.C.O. Macias, Centro Universitario de la Costa Sur, Mexico and a professional beekeeper, M.C.J. Espadas, Mexico. The volatile form of stingless propolis was hung near each subjects' bed to assist inhalation.

Subjects

All subjects were informed about the experimental procedures and gave their written consent before embarking on the study, although Iwate Prefectural University and Iwate University had no guidelines for the ethics commission during the experimental period of 2002. The experimental subjects were divided into 2 groups, who underwent different courses of treatment with propolis. 1) Thirty-four subjects (11 males and 23 females) were treated for 2 months with propolis (Experiment #1) at a nursing home in northeastern Japan. They ranged from 62 to 99 years old, with an average age of 80±8.7. Underlying diseases included 20 post-stroke subjects, 5 with senile dementia, 4 with cerebrovascular dementia, 1 with Parkinson's disease, 1 with subacute myelo-optico-neuropathy, 1 with cardiac failure, 1 with alimentary disease, and 1 with spinocerebellar degeneration. All subjects had cognitive functions and could communicate. The subjects in Experiment #2 were the same as described in Experiment #1, with the exception of 8 subjects who left the study area because their stay in the residence ended or due to a rearrangement of room assignments. Twenty-six subjects (6 males and 20 females) took part in this study. The age range was 62 to 99 years old with an average of 80.5±8.6.

Study design

Experiment #1: All treatments were performed for 2 months. Three rooms were each occupied by 4 people and 9 rooms had individual residents. A total of 21 subjects were exposed to 30 g of stingless honeybee propolis in a lace bag (about the size of a chicken's egg) that was hung from the bottom of a bedside lamp to avoid any annoyance (propolis group). Another group was exposed to 30 g of Binchô charcoal. This material, often used to diminish odor, is commonly hung from walls and appears similar to propolis. For the Binchô charcoal group, we used 2 rooms with 4 people each, giving a total of 8 people. The non-treated control group consisted of 4 people in 1 room and 1 individual who was housed in a single room.

Experiment #2: One month after Experiment #1, 16 subjects were re-treated with propolis by the same method and 10 subjects remained untreated for 1 month.

Measurement of natural killer (NK) cell activity and lymphocytes

For Experiment #1, blood samples were collected before and 2 months after each treatment. Samples were collected between 2 p.m. and 4 p.m. from June to November to avoid any circadian variations. According to routine studies (Ogata *et al.*, 2001; Pross and Maroun, 1984), NK cell activity was assayed by labeling K562 target cells with ^{51}Cr, and NK cells prepared from each subject were added as effectors. The ratio of effectors to target cells (E/T) was 20 : 1. After co-cultivation, the released ^{51}Cr from the target cells was counted to quantify NK cell activation. The lymphocyte phenotype of the subjects was determined by dual color analysis using a flow cytometer. The same trials were performed in the same way in Experiment #2.

Statistical analysis

McNemar's test was used for all statistical analyses. P values of below 0.05 were regarded as indicating statistical significance.

Results

Experiment # 1

1. Changes in NK cell activity

The NK cell activity of subjects treated with propolis was

Table 1. NK cell activity and lymphocytes before and after propolis treatment

Experimental period	Propolis group (n=21)		Charcoal group (n=8)		Control (n=5)		All subjects (n=34)	
	NK cell Activity[1)]	Lymphocytes	NK cell activity	Lymphocytes	NK cell activity	Lymphocytes	NK cell activity	Lymphocytes
Before treatment (end of June)	51.8±11.4	36.2±10.1	56.6±17.8	29.3±8.7	53.6±11.6	29.6±7.1	53.2±12.9	33.6±9.8
2 months after treatment (end of August)	39.9±9.9	34.7±7.8	48.7±18.1	31.7±8.9	46.6±24.5	24.0±5.0	43.0±14.7	32.2±8.5

[1)]Data are shown as average of NK cell activity or lymphocytes (%) for each group±standard deviation.

Table 2. Subsequent changes in NK cell activity as a result of each treatment

Treatment	n	Change	No. of subject[1)] in Normal	and %[2)] Out of Normal	Tatal No. of subjects
Propolis	21	Before	3(14)	18(86)	21
		After	11(52)	10(48)	21
Charcoal	8	Before	1(13)	7(87)	8
		After	2(26)	6(74)	8
Control	5	Before	1(20)	4(80)	5
		After	2(40)	3(60)	5

[1)]Data are shown as number of subjects in or out of the normal range in each treatment.
[2)]The percentage of subjects undergoing each treatment is shown in parentheses.

51.8±11.4% (max 74%, min 28%) before treatment and 39.9±9.9% (max 62%, min 25%) after 2 months' treatment, as shown in Table 1. NK cell activity in the Binchô charcoal-treated group was 56.6±17.8% (max 74%, min 18%) before and 48.7±18.1% (max 69%, min 15%) after treatment. That in the untreated control group was 53.6±11.6% (max 66%, min 37%) before and 46.6±24.5% (max 69%, min 14%) after 2 months. After propolis treatment, 14.3% of subjects showed increased NK cell activity, 9.5% showed no change, and 76.2% had decreased activity. In the Binchô charcoal group, 25% had increased activity and 75% had decreased activity. In the untreated control group, 40% had increased and 60% had decreased NK responses. Overall, there were more subjects with decreased NK cell activity after 2 months' treatment than subjects with increases.

2. Changes in NK cell activity as related to the normal value range of 18 to 40%

When the data were analyzed relative to normal NK values in all subjects, 5 (14.7%) had a normal range of NK cell activity before treatment and 13 (38.2%) were improved after treatment. In the propolis-treated group, 3/21 subjects (14.3%) were in the normal range before treatment and 11/21 subjects (52.4%) had improved NK cell activity after treatment (Table 2). Therefore, after propolis treatment, more than half of the subjects developed a normal range of NK cell activity. Only one subject had a normal range of NK cell activity both before and after treatment in the Binchô charcoal-exposed group. Similarly, a single subject within the untreated control group achieved normal NK levels during monitoring. In addition, 3 subjects in the propolis group maintained their normal NK cell activity before and after treatment, and 8 subjects acquired a normal range similar to those subjects.

In both the Binchô charcoal and the untreated control group, however, 1 subject in the normal range left the normal range within 2 months after either treatment or monitoring. As shown in Fig. 1, these data suggest that the NK cell activity of subjects treated with propolis tended to remain within the normal range, while both the charcoal and the untreated control groups tended to leave the normal range. According to McNemar's test, the propolis-treated group had significantly more subjects with a normal range of NK cell activity when compared with the other 2 groups ($p < 0.05$).

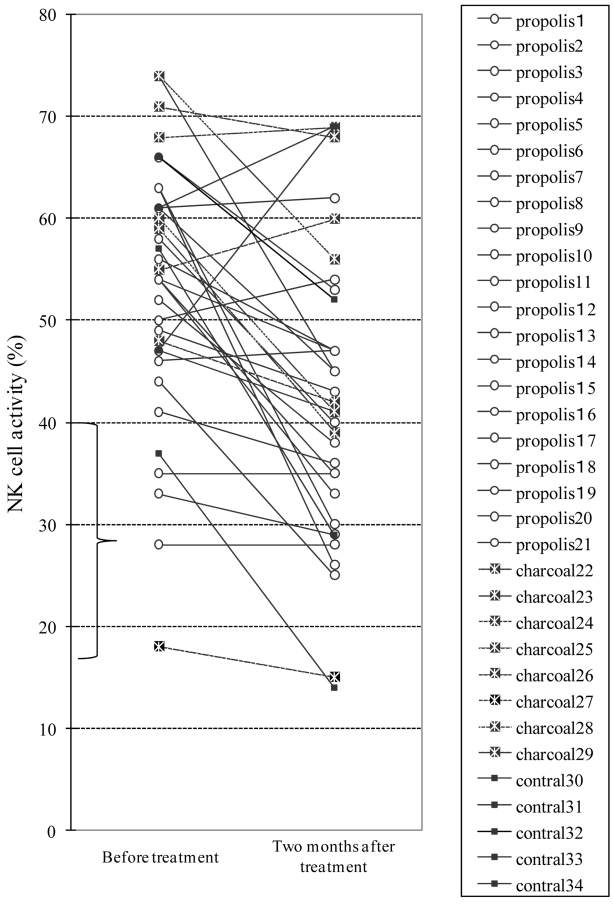

Fig. 1. Subsequent changes in NK cell activity of each subject before and after each treatment. Propolis was administered to 21 subjects (propolis 1~21, open circles), charcoal to 8 subjects (charcoal 22~29, crossed squares), and 5 subjects were not treated (control 30~34, closed squares). The NK cell activity (%) of all the subjects before and after 2 months' treatment is shown. The normal range of NK cell activity, 18 to 40%, is indicated in the bracket at the left.

Table 3. Changes in NK cell activity in the incidence of experiments # 1 and 2

Experimental period	NK cell activity (%)[1)]			
	Contiuous group (n=9)	Propolis removed group (n=10)	Charcoal propolis group (n=7)	All subjects (n=26)
Before treatment	49.7±12.2	53.6±11.6	56.6±17.8	53.1±13.2
2 months after treatment (end of August)	39.0±10.2	14.8±9.6	48.7±18.1	42.7±13.2
Continuous treatment (early November)	32.0±7.5	41.9±9.4	46.7±16.7	39.8±12.7

[1)]Date are shown as average of NK cell activity (%) of each group ±standard deviation.

3. Changes in the ratio of lymphocytes to total leucocytes
In the propolis-treated group, the average lymphocyte ratio was 36.2±10.1% (max 62%, min 17%) before treatment and 34.7±7.8% (max 52%, min 21%) after treatment (Table 1). The lymphocyte ratio of the Binchô charcoal-treated group increased from 29.3±8.7% (max 40%, min 19%) before treatment to 31.7±8.9% (max 43%, min 14%) after treatment. In the untreated control group, ratios were 29.6±7.1% (max 40%, min 23%) before and 24.0±5.0% (max 32%, min 18%) after 2 months' treatment. All the data shown were in the normal range (25~50%), and these results show that neither the propolis nor Binchô charcoal treatment affected lymphocyte ratios.

Experiment # 2

1. Subsequent changes in NK cell activity due to long-term treatment
To confirm whether propolis was exerting a long-term effect, 30 days after ceasing 2 months of continuous treatment, subjects were re-treated for 1 month. The results from Experiments #1 and 2 were combined into 3 groups (Table 3). Group 1 consisted of 9 subjects in the continuous group who received sequential courses of propolis. Group 2 (the propolis-removed group) consisted of 10 subjects treated with propolis for 2 months in Experiment #1, but who had received no further treatment. Group 3 (the charcoal-propolis treatment group) comprised 7 subjects treated with Binchô charcoal for 2 months in Experiment #1, who, 1 month later, were treated for a further 1 month with propolis in Experiment #2. As a result, only the continuous group had normal NK cell activity (32.0±7.5%), but the other groups were out of the normal range and showed no difference after the 2nd treatment.
2. Individual changes of NK cell activity
When the changes in NK cell activity in individuals in Experiment #2 were documented, 7 subjects acquired normal NK cell activity after the 1st treatment and 2 subjects had normal NK cell activity after the 2nd treatment (Fig. 2). This result suggests that continuous treatment, even in the presence of an interruption, indeed brought NK cell activity to values that were within the normal ranges.

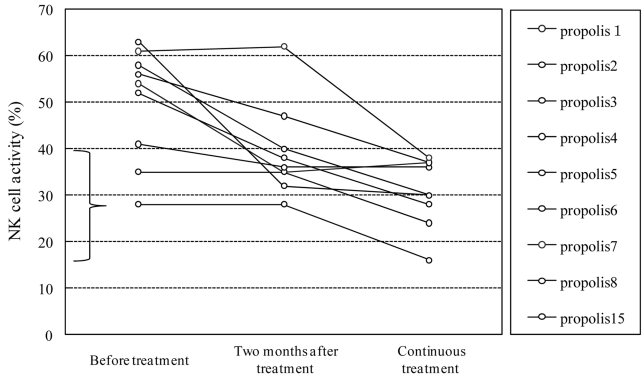

Fig. 2. Individual changes in NK cell activity in the continuous treatment of propolis volatiles. Nine subjects were continuously treated with propolis in both Experiments #1 and 2. The NK cell activity (%) of 9 subjects is shown before treatment, after treatment (Experiment #1), and after Experiment #2 (30 days after Experiment #1, 9 subjects were re-treated with propolis for 1 month). The normal range of NK cell activity, 18 to 40%, is indicated in the bracket at the left.

Discussion

It has often been claimed that the definition of the "normal range" of NK cell activity depends largely on the conditions of the experiment, such as different target cells, E/T ratio, reaction time, individual variation, and sex (Ogata *et al.*, 2001). When K562 cells are used as target cells and the E : T ratio is 20 : 1, the normal range is usually 18 to 40% (Kodama *et al.*, 2003). Since immune activity in elderly people is often measured by NK cell activity (Ogata *et al.*, 2001), we used this parameter to monitor the effects of propolis volatiles from the stingless honeybee *M. beecheii* on the immune activity of elderly residents of a nursing home. Both Experiments #1 and 2 showed that the volatile form of this propolis upregulated NK cell activity into the normal range, indicating that inhalation of the volatile substance that was simply placed in the room had a positive effect on the immune systems of elderly individuals.

When we also used propolis volatiles from the honeybee *A. mellifera* as well as in the present study, however,

its strong and unpleasant odor required a halt to the study (data not shown). Thus, these data indicate that the propolis sources used by *A. mellifera* and the stingless honeybee *M. beecheii* do not coincide. Pino *et al*. (2006) reported that α-pinene, β-pinene, trans-verbenol, α-copaene, β-boubonene, β-caryophyllene, spathulenol and caryophyllene oxide are found at higher levels in the propolis from the stingless honeybee *M. beecheii*. We also will provide further information for identifying the constituents of propolis volatiles from *M. beecheii* and *A.mellifera* in a future study.

It is of significant importance that an *in vivo* study of propolis extract from *A.mellifera* has been conducted on 47 healthy women and men to examine whether intake of propolis extract has any effect on some blood parameters (Jasprica *et al*., 2007). To our knowledge, the present study is the first demonstration of the effects of propolis supplementation from stingless honeybees on the immune system of human organisms. Elderly people tend to have a diminished immune capacity and tend to suffer from secondary infections such as bacterial pneumonia. Recovery is slow and subjects may either become bedridden or develop other syndromes. The propolis of stingless honeybees is therefore expected to improve the living environment and, as a result, has potential for the control of airborne infections such as influenza, a major threat to the health of elderly people, particularly residents of nursing homes (Nicolle *et al*., 1996).

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