ORIGINAL ARTICLE

A clinical pharmacological study of the potential beneficial effects of a propolis food product as an adjuvant in asthmatic patients

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ABSTRACT

The aqueous extract of propolis has been formulated as a nutritional food product and administered, as an adjuvant to therapy, to patients with mild to moderate asthma daily for 2 months in the framework of a comparative clinical study in parallel with a placebo preparation. The diagnosis of asthma was made according to the criteria of patient classification of the National Institutes of Health and Global Initiative for Asthma Management. At inclusion, the pulmonary forced expiratory volume in the first second (FEV_1) as a percentage of the forced vital capacity (FVC) was more than 80% in mild persistent cases, and between 60 and 80% in moderate persistent cases, showing an increase in the degree of reversibility of $> 15\%$ in FEV_1. All patients were on oral theophylline as controller therapy, none was receiving oral or inhaled corticosteroids, none had other comorbidities necessitating medical treatment, and all were from a middle-class community and had suffered from asthma for the last 2–5 years. Twenty-four patients received the placebo, with one drop-out during the study, while 22 received the propolis extract, with no drop-outs. The age range of the patients was 19–52 years; 36 were male and 10 female. The number of nocturnal attacks was recorded on a weekly basis, while pulmonary function tests were performed on all patients at the beginning of the trial, 1 month later and at the termination of the trial. Immunological parameters, including various cytokines and eicosanoids known to play a role in asthma, were measured in all patients at the beginning of the trial and 2 months later. Analysis of the results at the end of the clinical study revealed that patients receiving propolis showed a marked reduction in the incidence and severity of nocturnal attacks and improvement of ventilatory functions. The number of nocturnal attacks dropped from an average of 2.5 attacks per week to only 1. The improvement in pulmonary functions was manifested as a nearly 19% increase in FVC, a 29.5% increase in FEV_1, a 30% increase in peak expiratory flow rate (PEFR), and a 41% increase in the forced expiratory flow rate between 25 and 75% of the vital capacity (FEF_{25-75}). The clinical improvement was associated with decreases by 52, 65, 44 and 30%, respectively, of initial values for the pro-inflammatory cytokines tumor necrosis factor (TNF)-α, ICAM-1, interleukin (IL)-6 and IL-8, and a 3-fold increase in the 'protective' cytokine IL-10. The levels of prostaglandins E_2 and $F_{2\alpha}$ and leukotriene D_4 were decreased significantly to 36, 39, and 28%, respectively, of initial values. Patients on the placebo preparation showed no significant improvement in ventilatory functions or in the levels of mediators. The findings suggest that the aqueous propolis extract tested

is potentially effective as an adjuvant to therapy in asthmatic patients. The benefits may be related to the presence in the extract of caffeic acid derivatives and other active constituents.

INTRODUCTION

Propolis, or bee-glue, a crude resinous natural product elaborated by honey bees, contains a mixture of biologically active constituents composed chemically of terpenes, cinnamic acid, caffeic acid and their esters, amino acids and flavonoids [1,2]. Propolis extracts have been used widely in folk medicine for the management of various disorders. The aqueous extract of propolis (AEP) was previously shown to possess a variety of pharmacological activities including antibacterial [3], antiviral [4,5] and anti-inflammatory [6,7] activities. It has also been reported that AEP guards against cellular damage induced by ionizing radiation [8,9], an effect that is possibly related to its anti-inflammatory as well as to its free radical scavenging and antioxidant properties [2,10]. The anti-inflammatory activity was shown to be associated with appreciable inhibition of prostaglandins (PGs) and leukotrienes (LTs) [7], an effect that prompted us to speculate that it might be of benefit in the management of asthma, as that condition may also be regarded as an inflammatory state associated with the release of these mediators, among others. Pilot clinical trials in 10 human asthmatic volunteers using the AEP in tablet form (2–3 tablets per day containing 0.34 mg of aromatic organic acid/tablet) showed a marked improvement in pulmonary functions and in the severity and frequency of attacks (unpublished observations). The present study has therefore been designed as a comparative placebo-controlled study in order to assess whether AEP used as a nutritional supplement in the form of a milk-based food product improves the clinical status of asthmatic patients.

MATERIALS AND METHODS

Test product

A 13% solution of AEP was supplied by Propharma (Stenlose, Denmark). The product was prepared by aqueous decoction of crude propolis, collected from Denmark, China, Uruguay and Brazil, and standardized to contain not less than 0.05% of organic aromatic acids, mainly caffeic, ferulic, iso-ferulic, cinnamic and 3,4-dimethoxy-cinnamic acids in addition to trace amounts of various flavonoids. The aqueous extract was first concentrated then spray-dried under high pressure before being incorporated into the milk formula. The sachets were intended to be given suspended in water as a milk drink orally once a day for a 2-month period. Silver- and white-coded sachets containing the AEP and a placebo preparation, respectively, were provided as milk products by Friesland-Coberco Dairy Foods (Meppel, the Netherlands). The contents of the two types of sachet were identical in taste, smell, colour, and packaging volume, as approved by a special test panel set up by the firm. Each silver sachet was divulged at the termination of the study to contain active constituents equivalent to those present in 2 mL of AEP (13% solution).

Subjects

Forty-six human volunteer patients (36 males and 10 females), with an age range of 19–52 years, suffering from mild to moderate asthma for the last 2–5 years, were enrolled in the present study. The clinical asthma diagnosis was made according to the standard criteria of patient classification of the National Institutes of Health and Global Initiative for Asthma Management in 1998 [11]. They were admitted to Koubri El-Koubbeh Army Hospital in Cairo or to the Chest Disease Unit at the Kasr El-Aini Hospital, Faculty of Medicine, Cairo University. All participating individuals were briefed on the study in question and on the possible side-effects and risks involved. They gave their written informed consent at the beginning of the trial. The whole study protocol was approved and followed up by a special medical Ethical Committee set up for the purpose of this investigation by the Faculty of Medicine, Cairo University, in accordance with international guidelines.

Exclusion criteria

These included the following: (i) any patient showing adverse effects during the treatment period; (ii) any patient having to resort to excessive rescue medication (patients requiring more than two puffs of salbutamol, a selective β_2 adrenoceptor agonist, 100 μg/puff, more

than four times a day, as these patients were considered at high risk of acute exacerbation of asthmatic attacks and would have had to take corticosteroids); (iii) any patient taking any corticosteroids within the last 2 months; (iv) any patient with a history of allergic manifestations to drugs, such as rashes or fever; (v) any patient who had suffered from acute severe asthma necessitating hospitalization during the last 6 months, and (vi) any patient with other comorbidities, such as diabetes or hypertension, necessitating medical treatment.

Inclusion criteria

These included the following: (i) Patients presenting with a complaint of repeated attacks of dyspnoea and wheezy chest, and maintained on an oral theophylline slow-release preparation, 200 mg/day. They were diagnosed as having mild to moderate asthma according to the standard criteria mentioned above. The pulmonary forced expiratory volume in the first second (FEV_1) as a percentage of the forced vital capacity (FVC) was more than 80% in mild persistent cases, and between 60 and 80% in moderate persistent cases, showing an increase in the degree of reversibility of > 15% in FEV_1. Whenever necessary, they were given salbutamol, as rescue medication, by meter dose inhalation (two puffs, each supplying 100 μg, not more than four times a day). (ii) Patients had received no systemic or local corticosteroid therapy for at least 2 months, and no other medication of any sort for at least 2 weeks before admission to the study. (iii) Patients had no history of acute severe asthma during the last 6 months prior to the study.

Each asthmatic patient referred to the hospitals was subjected to: (i) a complete medical history, particularly relating to the date of onset of the asthma complaint, the number of nocturnal attacks per week and precipitating factors that aggravated or prompted the attacks, and (ii) a thorough physical examination to exclude any patient with signs of irreversible chronic obstruction of airways or infections. The forced expiratory volume test was carried out by asking the patients to breathe slowly and tidally and then to take a full inspiration followed by a forcible, fast expiration, and lastly to take a full, deep, forcible inspiration. After three trials, two puffs (50 μg each) of meter-dosed salbutamol inhalation were given and the test was repeated after 10 min. This procedure abolished signs of airway obstruction if it was reversible, but not if it was irreversible. The reversibility test was carried out on all patients in the trial, to ensure that none of them suffered from irreversible airway obstruction.

Study protocol

Asthmatic patients were randomly allocated to two groups, such that age, sex, and asthma severity were nearly evenly distributed in the two groups. One group received the white sachets (24 individuals) and the other received the silver ones (22 individuals). The sachets were given to the patients on a daily basis as an adjuvant to the regular therapeutic medication. Before the initiation of the treatment, the pulmonary ventilatory function tests were performed (see below). Blood samples were also collected for the estimation of certain inflammatory mediators and immunological factors. The pulmonary ventilatory functions were again assessed after 1 and 2 months of the respective treatment, while blood samples were collected again after 2 months. In addition, the patients were examined weekly for any subjective improvement or worsening of their condition. The number of nocturnal attacks per week and their duration were also recorded. The daily consumption of rescue medication was also recorded and did not exceed four times per day of salbutamol inhalation (two puffs, each of 100 μg).

Pulmonary ventilatory function tests

Pulmonary ventilatory functions, mainly flow-volume loop manoeuvres, were investigated before and after two puffs of inhaled salbutamol using a pneumotechograph, V Max (Sensor Medics 2200 NV, Dilthoven, the Netherlands) aided by an IBM (PS2) computer. The parameters studied were FVC, FEV_1, the peak expiratory flow rate (PEFR) and the forced expiratory flow between 25 and 75% of the vital capacity (FEF_{25-75}). All parameters were measured three times before and three times after bronchodilator treatment. The maximum predicted value for each parameter was computed as a percentage of the predicted value for a person of the same age, race, sex, height, and body weight according to the requirements of the automated system. The FVC, FEV_1 and PEFR were chosen to reflect the function of the major airways, while FEF_{25-75} was selected to reflect the function of the small airways.

Estimation of inflammatory mediators and immunological factors in blood

Peripheral venous blood from the forearm was withdrawn from each patient by venipuncture into an ice-cold nonheparinized tube, and the serum was separated by centrifugation at 1000 ***g*** for 15 min. The collected sera were used for the estimation of certain inflammatory mediators, namely, PGE_2, $PGF_{2\alpha}$, and LTs deter-

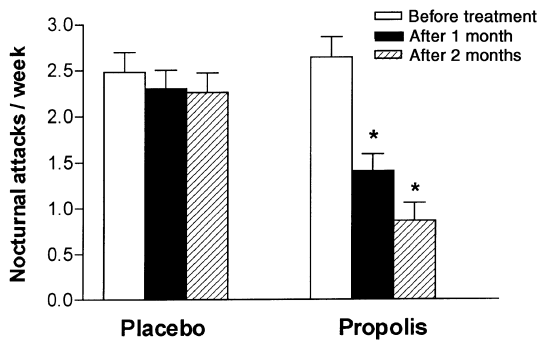

Figure 1 Number of nocturnal attacks per week in asthmatic patients taking placebo-containing (white) sachets ($n = 23$) and aqueous extract of propolis-containing (silver) sachets ($n = 22$). The sachets were prepared as milk products intended to be given suspended in water as a drink orally once a day for 2 months. The sachets were given to the asthmatic patients as an adjuvant to the regular therapeutic medication. Results are expressed as the mean values ± SEM. *Significant difference at $P < 0.001$.

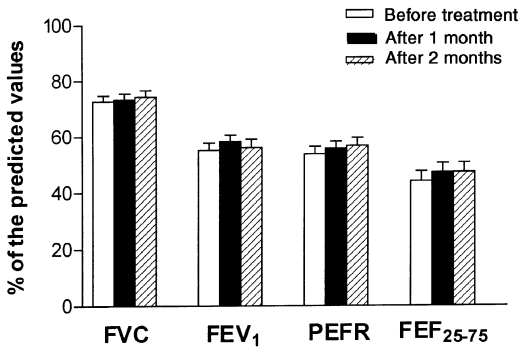

Figure 2 Effects of placebo-containing (white) sachets on the pulmonary ventilatory functions (the flow-volume loop manoeuvres) in asthmatic patients ($n = 23$). The tested parameters were the forced vital capacity (FVC), the forced expiratory volume in the first second (FEV_1), the peak expiratory flow rate (PEFR) and the forced expiratory flow between 25 and 75% of the vital capacity (FEF_{25-75}). The sachets were prepared as milk products intended to be given suspended in water as a drink orally once a day for 2 months. The product was given to the asthmatic patients as an adjuvant to the regular therapeutic medication. Results are expressed as mean percentages of the predicted values for a person of the same age, race, sex, height, and body weight. Vertical lines indicate SEM.

mined collectively as LTD_4, as well as for the measurement of certain immunological factors, namely, tumour necrosis factor (TNF)-α, intercellular adhesion molecule (ICAM)-1, interleukin (IL)-6 , IL-8 and IL-10. PGs and LTs were measured by radioimmunoassay using the respective kits purchased from Amersham International (Amersham, UK), while TNF-α, ICAM-1 and the various ILs were assessed by the enzyme-linked immunosorbent assay technique using the respective kits supplied by Predicta, Genzyme Diagnostics (Cambridge, MA, USA).

Statistical analysis

Data are expressed as means ± SEM. The results before and after the administration of either type of sachet were analysed statistically using Student's *t*-test for paired data. GRAPHPAD INSTAT software version 2.04 (Graphpad Software Inc., San Diego, CA, USA) was used to analyse the differences between the means. A difference was considered statistically significant at $P = 0.05$.

RESULTS

Placebo (white-coded) sachets

Twenty-four asthmatic patients were started on the placebo sachets, but one patient did not feel he needed to continue the study and thus dropped out during the treatment. The sachets were given to the patients on a daily basis as an adjuvant to the regular therapeutic medication. Neither the incidence and intensity of nocturnal attacks (*Figure 1*) nor the tested pulmonary ventilatory functions (*Figure 2*) changed markedly during the 2-month treatment period. None of the patients, however, showed any worsening of their condition. In general, there was little change in the serum levels of the measured immunological factors and inflammatory mediators. The serum levels of TNF-α (*Figure 3*), ICAM-1

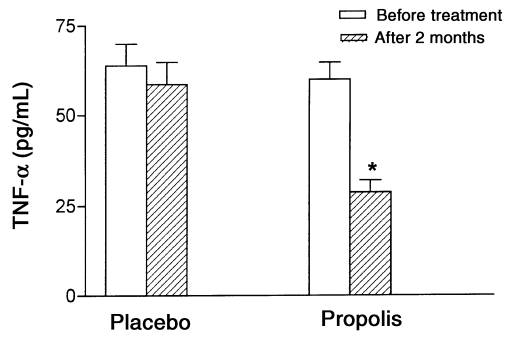

Figure 3 Levels of tumour necrosis factor (TNF)-α in the serum of asthmatic patients taking placebo-containing (white) sachets ($n = 23$) and aqueous extract of propolis-containing (silver) sachets ($n = 22$). The sachets were prepared as milk products intended to be given suspended in water as a drink orally once a day for 2 months. The sachets were given to the asthmatic patients as an adjuvant to the regular therapeutic medication. Results are expressed as the mean values ± SEM. *Significant difference at $P = 0.0001$.

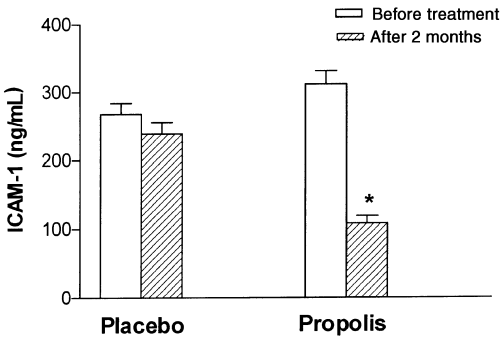

Figure 4 Levels of intercellular adhesion molecule-1 (ICAM-1) in the serum of asthmatic patients taking placebo-containing (white) sachets ($n = 23$) and aqueous extract of propolis-containing (silver) sachets ($n = 22$). The sachets were prepared as milk products intended to be given suspended in water as a drink orally once a day for 2 months. The sachets were given to the asthmatic patients as an adjuvant to the regular therapeutic medication. Results are expressed as the mean values ± SEM. *Significant difference at $P = 0.0001$.

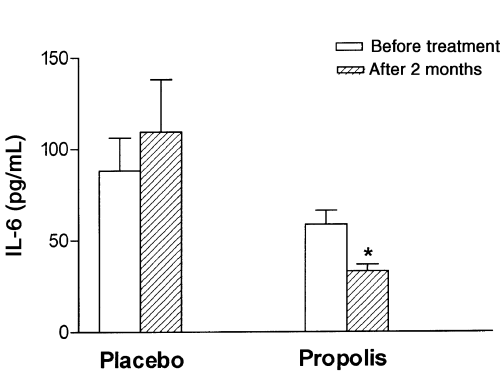

Figure 5 Levels of interleukin (IL)-6 in the serum of asthmatic patients taking placebo-containing (white) sachets ($n = 23$) and aqueous extract of propolis-containing (silver) sachets ($n = 22$). The sachets were prepared as milk products intended to be given suspended in water as a drink orally once a day for 2 months. The sachets were given to the asthmatic patients as an adjuvant to the regular therapeutic medication. Results are expressed as the mean values ± SEM. *Significant difference at $P = 0.002$.

(*Figure 4*), IL-6 (*Figure 5*), IL-8 (*Figure 6*) and LTs (*Figure 7*) showed no significant alterations. The serum levels of IL-10 and PGs were either slightly raised, slightly lowered, or remained unchanged, such that on average, the level of IL-10 was raised to a small extent while that of PGs was lowered to only a small extent. *Figures 8, 9* and *10* illustrate collectively the overall pattern of change in the serum levels of IL-10, PGE_2 and $PGF_{2\alpha}$, respectively. The immunological findings seem to correlate well with

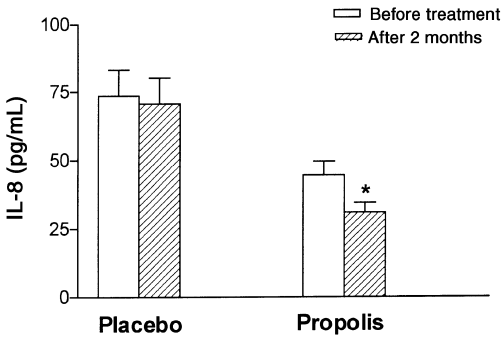

Figure 6 Levels of interleukin (IL)-8 in the serum of asthmatic patients taking placebo-containing (white) sachets ($n = 23$) and aqueous extract of propolis-containing (silver) sachets ($n = 22$). The sachets were prepared as milk products intended to be given suspended in water as a drink orally once a day for 2 months. The sachets were given to the asthmatic patients as an adjuvant to the regular therapeutic medication. Results are expressed as the mean values ± SEM. *Significant difference at $P = 0.008$.

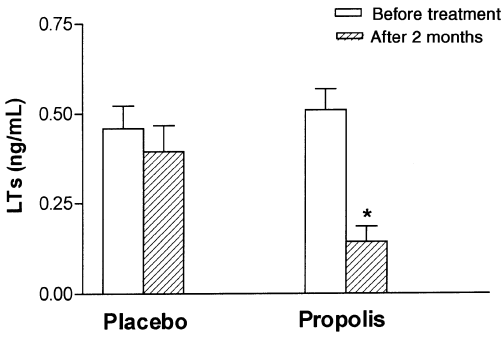

Figure 7 Levels of leukotrienes (LTs) in the serum of asthmatic patients taking placebo-containing (white) sachets ($n = 23$) and aqueous extract of propolis-containing (silver) sachets ($n = 22$). The sachets were prepared as milk products intended to be given suspended in water as a drink orally once a day for 2 months. The sachets were given to the asthmatic patients as an adjuvant to the regular therapeutic medication. Results are expressed as the mean values ± SEM. *Significant difference at $P = 0.0001$.

the clinical results, which revealed that these patients did not benefit much from treatment. There was no change in the requirements of the patients for rescue medication (two puffs of 100 μg salbutamol each, 1–4 per day as necessary) throughout the trial.

Propolis (silver-coded) sachets

Twenty-two asthmatic individuals were included in this treatment regimen, namely, propolis sachets for 2 months. The sachets were given to the patients on a daily basis as an adjuvant to the regular therapeutic

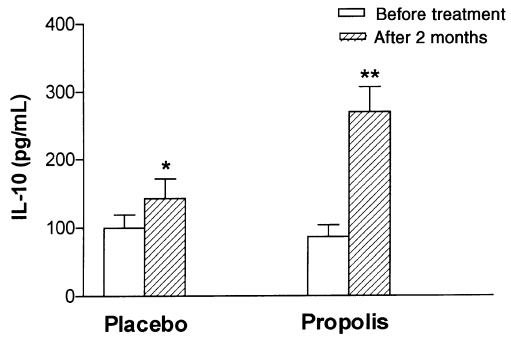

Figure 8 Levels of interleukin-10 (IL-10) in the serum of asthmatic patients taking placebo-containing (white) sachets ($n = 23$) and aqueous extract of propolis-containing (silver) sachets ($n = 22$). The sachets were prepared as milk products intended to be given suspended in water as a drink orally once a day for 2 months. The sachets were given to the asthmatic patients as an adjuvant to the regular therapeutic medication. Results are expressed as the mean values ± SEM. *Significant difference at $P = 0.02$. **Significant difference at $P = 0.0001$.

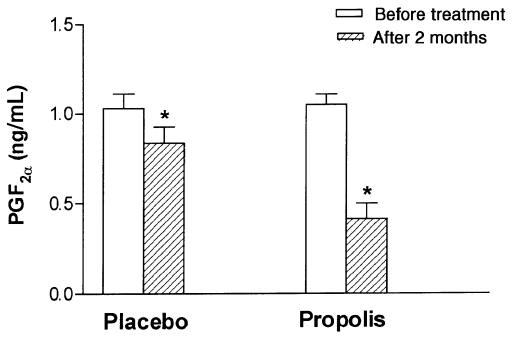

Figure 10 Levels of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) in the serum of asthmatic patients taking placebo-containing (white) sachets ($n = 23$) and aqueous extract of propolis-containing (silver) sachets ($n = 22$). The sachets were prepared as milk products intended to be given suspended in water as a drink orally once a day for 2 months. The sachets were given to the asthmatic patients as an adjuvant to the regular therapeutic medication. Results are expressed as the mean values ± SEM. *Significant difference at $P = 0.0001$.

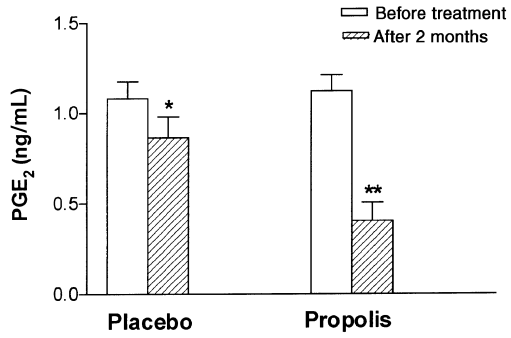

Figure 9 Levels of prostaglandin E_2 (PGE_2) in the serum of asthmatic patients taking placebo-containing (white) sachets ($n = 23$) and aqueous extract of propolis-containing (silver) sachets ($n = 22$). The sachets were prepared as milk products intended to be given suspended in water as a drink orally once a day for 2 months. The sachets were given to the asthmatic patients as an adjuvant to the regular therapeutic medication. Results are expressed as the mean values ± SEM. *Significant difference at $P = 0.001$. **Significant difference at $P = 0.0001$.

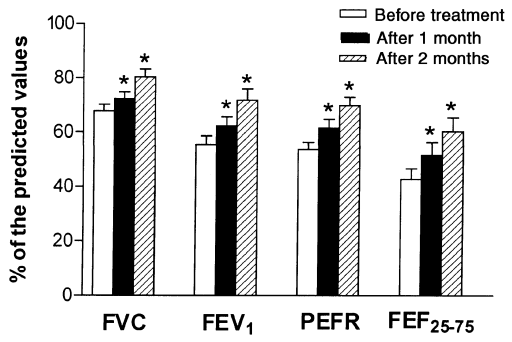

Figure 11 Effects of aqueous extract of propolis-containing (silver) sachets on pulmonary ventilatory functions (the flow-volume loop manoeuvres) in asthmatic patients ($n = 22$). The tested parameters were the forced vital capacity (FVC), the forced expiratory volume in the first second (FEV_1), the peak expiratory flow rate (PEFR) and the forced expiratory flow between 25 and 75% of the vital capacity (FEF_{25-75}). The sachets were prepared as milk products intended to be given suspended in water as a drink orally once a day for 2 months. The product was given to the asthmatic patients as an adjuvant to the regular therapeutic medication. Results are expressed as mean percentages of the predicted values for a person of the same age, race, sex, height, and body weight. Vertical lines indicate SEM. *Significant difference at $P = 0.0001$.

medication. A definite reduction in the frequency and severity of nocturnal attacks was already evident after the first month, and was even more pronounced after 2 months (*Figure 1*). Nearly half the patients no longer experienced nocturnal attacks. Furthermore, many patients improved to the extent that they required fewer doses of their normal medication. The tested pulmonary ventilatory functions were also markedly improved. Such improvement was manifested in both the large and small airway functions as determined by increases in the values of FVC by 6.8 and 18.7%, FEV_1 by 12.3 and 29.5%, PEFR by 14.4 and 29.8%, and FEF_{25-75} by 20.9 and 40.9% after 1 and 2 months, respectively (*Figure 11*). The values for FEV_1 at inclusion in the study were assessed as a percentage of FVC, whereas after treatment FEV_1 was

computed as a percentage of the predicted value. The improvement in FEV_1 was calculated for each patient in comparison to his or her own previous data. The clinical findings were accompanied by beneficial alterations in the serum levels of the measured immunological factors and inflammatory mediators. Thus, there was a more than 3-fold increase in the level of IL-10 (*Figure 8*) associated with decreases in the levels of TNF-α (*Figure 3*), ICAM-1 (*Figure 4*), IL-6 (*Figure 5*) and IL-8 (*Figure 6*) by 52, 65, 44 and 30%, respectively. The serum levels of PGE_2 (*Figure 9*), $PGF_{2\alpha}$ (*Figure 10*) and LTs (*Figure 7*) were dramatically reduced to 36, 39 and 28% of their initial values, respectively. The need for rescue medication was reduced in nearly all patients from 1–4 times a day to 0–2 times only.

DISCUSSION

It is well established that peptide mediators, such as cytokines and growth factors, orchestrate and perpetuate the chronic inflammatory features of diseases such as bronchial asthma [12]. Our present findings show a close association between the clinical findings and the changes in immunological parameters measured. TNF-α is a potent immunomodulator cytokine, whose levels are elevated in many inflammatory states, including bronchial asthma. Many studies have shown that bronchoalveolar lavage fluid from patients with symptomatic asthma contains significantly elevated levels of TNF-α [13,14]. The elevated values play a key role in the activation and production of other pro-inflammatory cytokines, including IL-1 [15], IL-6 [16] and IL-8 [17], as well as ICAM-1 expression [18]. The release of PGs may ensue as a result of the release of the cytokines IL-1 and/or IL-8 [17]. The values of TNF-α were either not changed or indeed often raised in patients treated with the white-coded (placebo) sachets. However, in patients treated with the silver-coded (propolis) sachets (with the exception of two), there was a tendency for the TNF-α values to decrease after 2 months of treatment, indicating a process of downregulation of the expression of most of the pro-inflammatory cytokines and adhesion molecules on the surfaces of neutrophils, bronchial mucosal cells, and endothelial cells. The changes in TNF-α levels correlate well with the improvement in pulmonary functions of these patients.

ICAM-1 expression is upregulated on inflamed bronchial epithelium and appears to be important in the pathogenesis of airway hyperresponsiveness and asthma [18–20]. It is an important early marker of immune activation and immune response [21]. Patients taking the white sachets showed no improvement in this parameter, while patients taking the silver sachets showed a significant reduction in ICAM-1, to nearly half of the original values, indicating the efficacy of the silver sachets (containing AEP) in damping the inflammatory process in the bronchial tree.

An important role of IL-6 is to stimulate immunoglobulin production through activation of B lymphocytes [22]. It also stimulates the release of acute phase proteins from hepatocytes, which are considered of value in nonspecific host defence mechanisms against infection [23]. The only nearly consistent lowering of this factor was seen in the serum of patients taking the silver-coded sachets, possibly indicating a partial cessation of the inflammatory process, and again correlating well with the overall clinical picture.

The implication of IL-8 in inflammatory processes and asthma is also well established. It activates polymorphonuclear leukocytes (PMNs), leading to the generation of superoxide anion radicals and LTs [24–26]. The cytokine is chemotactic for activated T lymphocytes and basophils [27], which are then stimulated to release the inflammatory mediators histamine and LTs [28,29]. It also causes neutrophils to migrate to the luminal side of airway epithelial cells in vivo [30]. In the current investigation, patients on the white sachets showed little change, while those on the silver sachets showed mainly a reduction in this cytokine.

Some cytokines have been described which inhibit the production of other cytokines that are generally regarded as pro-inflammatory such as TNF-α, IL-1β, IL-6 and IL-8 [31]. One such 'antagonist cytokine' is IL-10. IL-10 inhibits the production of cytokines by murine Th1 lymphocytes [32] and is believed to play a role in inhibiting the delayed type of hypersensitivity response [33,34] and to suppress macrophage function and the synthesis of many cytokines, including IL-1, IL-6, IL-8 and TNF-α [35–38]. Effective anti-inflammatory treatment would therefore be expected to elevate the serum level of IL-10 with consequent inhibition of TNFα, as well as IL-6, IL-8, PGs, and LTs. In this study, IL-10 levels were not markedly affected by treatment with the white-coded sachets, but were significantly raised in patients taking the silver sachets. This correlates well with the findings on TNF-α and other mediators, as well as with the overall clinical improvement of these patients.

Prostanoids are produced under both physiological and pathophysiological conditions by all cell types present in the lung and are known to modulate various

airway functions including airway and vascular tone, cell proliferation, plasma exudation, inflammatory cell recruitment and activity, cytokine release and mucus secretion. Thromboxane A_2 has been implicated in the hyperresponsiveness of airway smooth muscle, which is often associated with allergen challenge [39]. In contrast, PGE_2 is a potent inhibitor of bronchoconstriction induced by allergens [40] and exercise [41,42], while asthmatic patients are particularly sensitive to $PGF_{2\alpha}$ which can cause intense bronchospasm. As well as eliciting functional responses to prostanoids through activation of cell surface receptors, airway smooth muscle cells themselves can produce prostanoids which may then act in an autocrine fashion [43,44]. The response of asthmatics to prostaglandins varies widely and depends not only on the relative concentration of the different PGs, but also on patient sensitivity.

In the present study patients taking the white sachets showed little change in the eicosanoids tested, apart from a small reduction of PGE_2 levels with no clinical relevance. In contrast, patients taking the silver sachets showed a significant reduction in PGE_2, $PGF_{2\alpha}$ and LTs, indicating that the constituents inhibited eicosanoid production at the beginning of the arachidonic acid cascade. The clinical improvement of patients taking the silver sachets seems therefore to be related more to inhibition of $PGF_{2\alpha}$ and LTs than to inhibition of PGE_2.

AEP was previously found to inhibit the immunological release of PGs and LTs from perfused sensitized guinea-pig lung [7] and to reduce carrageenan-oedema and inhibit lipid peroxidation in irradiated animals [9]. The extract was also found to have free radical scavenging properties in vitro [2,10]. The effect of propolis, in general, was attributed to its content of biologically active substances, which include flavonoids and caffeic acid esters. Caffeic acid itself as well as its derivative caffeic acid phenethyl ester have been shown to inhibit the biosynthesis of LTs [45], to inhibit lipid peroxidation [46] and to suppress the oxidation burst of PMNs [45]. The flavonoid content of propolis includes chrysine, apigenin, kaempferol, acacetin, galangin and quercetin [3,4]. According to Baumann et al. [47] galangin in low concentrations inhibits both cyclooxygenase and lipoxygenase activity. Quercetin and kaempferol are potent inhibitors of PGE_2 [48] and quercetin decreases the release of lysosomal enzyme from human PMNs in vitro [49]. However, the aqueous extract of propolis hardly contains any flavonoids, and the observed effects cannot be attributed to them.

The above effects are intricately interrelated with the observed beneficial findings on the studied immunological parameters. The overall reduction in the level of the pro-inflammatory cytokines, and the rise in the level of the 'protective' ones, largely contributed to the improved ventilatory functions seen in patients taking the silver sachets containing AEP. It was interesting to note that patients on AEP were found to resort much less to rescue medication by the end of the trial than those on placebo. Furthermore, the present study shows a close correlation between the clinical manifestations and pulmonary functions of asthmatic patients and relevant biological parameters.

In conclusion, in a comparison of the effect of the aqueous propolis extract (silver sachets), used as a nutritional adjuvant to antiasthmatic therapy, with that of a placebo (white sachets), it seemed that the former did confer definite advantages in reducing the frequency and severity of attacks, possibly by improving the immunological reactivity of the patients. However, patients treated with a placebo did not improve to any appreciable extent, yet the product was well tolerated. There was no significant reduction in the frequency or severity of the nocturnal attacks, nor was there a tangible improvement in ventilatory functions. The overall beneficial effect in patients taking the silver sachets was accompanied by a real improvement in ventilatory functions pertaining to both the large and small airway passages, as well as by a reduction in the need for rescue medication. The aqueous extract of propolis may thus find a place as a nutritional supplement in the routine management of bronchial asthma.

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REFERENCES

1 Walker P., Crane E. Constituents of propolis. Apidologie (1987) **18** 327–334.

2 Volpert R., Elstner E.F. Biochemical activities of propolis extracts. I. Standardization and antioxidative properties of ethanolic and aqueous derivatives. Z. Naturforsch. (1993) **48** 851–857.

3 Takaisi-Kikuni N.B., Schilcher H. Electron microscopic and microcalorimetric investigations of the possible mechanism of the antibacterial action of a defined propolis provenance. Planta Med. (1994) **60** 222–227.

4 Debiaggi M., Tateo F., Pagani L., Luini M., Romero E. Effects of propolis flavonoids on virus infectivity and replication. Microbiologica. (1990) **13** 207–213.
5 Amoros M., Lurton E., Boustie J., Girre L., Sauvager F., Cormier M. Comparison of the anti-herpes simplex virus activities of propolis and 3-methyl-but-2-enyl caffeate. J. Nat. Prod. (1994) **57** 644–647.
6 Metzner J., Bekemeier H., Weber F.G. Influence of flavonoids on capillary permeability, carrageenin edema and histamine and PGE_2 spasms. Agents Actions (1982) **10** (Suppl.) 129–134.
7 Khayyal M.T., El-Ghazaly M.A., El-Khatib A.S. Mechanisms involved in the antiinflammatory effect of propolis extract. Drugs Exp. Clin. Res. (1993) **19** 197–203.
8 Scheller S., Wilczok T., Imielski S., Krol W., Gabrys J., Shani J. Free radical scavenging by ethanol extract of propolis. Int. J. Rad. Biol. (1990) **57** 461–465.
9 El-Ghazaly M.A., Khayyal M.T. The use of aqueous propolis extract against radiation-induced damage. Drugs Exp. Clin. Res. (1995) **21** 229–236.
10 Pascual C., Gonzalez R., Torricella R.G. Scavenging action of propolis extract against oxygen radicals. J. Ethnopharmacol. (1994) **41** 9–13.
11 National Institutes of Health. Global Strategy for Asthma Management and Prevention. NIH Publication No. 023659. National Heart, Lung, and Blood Institute, NIH, USA. 1998.
12 Barnes P.J. Cytokines as mediators of chronic asthma. Am. J. Respir. Crit. Care Med. (1994) **150** S42–S49.
13 Mattoli S., Mattoso V.L., Solopertro M., Allegra L., Fasoli A. Cellular and biochemical characteristics of bronchoalveolar lavage fluid in symptomatic non-allergic asthma. J. Allergy Clin. Immunol. (1991) **87** 794–802.
14 Broide D.H., Lotz M., Cuomo A.J., Coburn D.A., Federman E.C., Wasserman S.I. Cytokines in symptomatic asthma airways. J. Allergy Clin. Immunol. (1992) **89** 958–967.
15 Dinarello C.A., Ikejima T., Warner S.J.C. et al. Interleukin 1 induces interleukin1. I. Induction of circulating interleukin1 in rabbits *in vivo* and in human mononuclear cells *in vitro*. J. Immunol. (1987) **139** 1902–1910.
16 Schindler R., Mancilla J., Andres S., Ghorbani R., Clark S.C., Dinarello C.A. Correlation and interactions in the production of interleukin-6 (IL-6), and tumour necrosis factor (TNF) in human blood mononuclear cell: IL-6 suppresses IL-1 and TNF. Blood (1990) **75** 40–47.
17 Streiter R.M., Kunkel S.L., Showell H.J. et al. Endothelial cell gene expression of a neutrophil chemotactic factor by TNFα, LPS, and IL-1α. Science (1989) **243** 1467–1469.
18 Ohkawara Y., Yamauchi K. Expression of adhesion molecules in bronchial asthma. Nihon Kyobu Shikkan Gakkai Zasshi (1993) **31** 125–131.
19 Nakamura Y. Infiltration of inflammatory cells and expression of adhesion molecules in bronchial mucosa of patients with asthma. Nihon Kyobu Shikkan Gakkai Zasshi (1996) **34** 1317–1325.
20 Bagnato G., Gulli S., Altavilla D. et al. Circulating adhesion molecules in bronchial asthma. J. Invest. Allergol. Clin. Immunol. (1998) **8** 105–108.
21 Bentley A.M., Durham S.R., Robinson D.S. et al. Expression of endothelial and leukocyte adhesion molecules, intercellular adhesion molecule-1, E-selectin, and vascular cell adhesion molecule-1 in the bronchial mucosa in steady-state and allergen-induced asthma. J. Allergy Clin. Immunol. (1993) **92** 857–868.
22 Van Snick J. Interleukin-6: an overview. Annu. Rev. Immunol. (1990) **8** 253–278.
23 Castell J.V., Gomez-Lechon M.J., David M., Hirano T., Kishimoto T., Heinrich P.C. Recombinant human interleukin-6 (IL-6/BSF-2/HSF) regulates the synthesis of acute phase proteins in human hepatocytes. FEBS Lett. (1988) **232** 347–350.
24 Baggiolini M., Walz A., Kunkel S.L. Neutrophil-activating peptide-1/interleukin 8, a novel cytokine that activates neutrophils. J. Clin. Invest. (1989) **84** 1045–1049.
25 Carveth H.J., Bohnsack J.F., McIntyre T.M., Baggiolini M., Prescott S.M., Zimmermann G.A. Neutrophil activating factor (NAF) induces polymorphonuclear leukocyte adherence to endothelial cells and to subendothelial matrix proteins. Biochem. Biophys. Res. Commun. (1989) **162** 387–393.
26 Schröder J.M. The monocyte-derived neutrophil activating peptide (NAP/Interleukin 8) stimulates human neutrophil arachidonate-5-lipoxygenase, but not the release of cellular arachidonate. J. Exp. Med. (1989) **170** 847–863.
27 Leonard E.J., Skeel A., Yoshimura T., Noer K., Kutvirt S., Van Epps D. Leukocyte specificity and binding of human neutrophil attractant/activation protein-1. J. Immunol. (1990) **144** 1323–1330.
28 Bischoff S.C., Baggiolini M., de Weck A.L., Dahinden C.A. Interleukin 8-inhibitor and inducer of histamine and leukotriene release in human basophils. Biochem. Biophys. Res. Commun. (1991) **179** 628–633.
29 Kuna P., Reddigari S.R., Kornfeld D., Kaplan A.P. IL-8 inhibits histamine release from human basophils induced by histamine-releasing factors, connective tissue activating peptide III, and IL-3. J. Immunol. (1991) **147** 1920–1924.
30 Johrens P.G., Richman-Eisenstat J.B.Y., Housset B.P. et al. Interleukin-8 induces neutrophil accumulation but not protease secretion in the canine trachea. Lung Cell. Mol. Physiol. (1992) **7** L708–L713.
31 Poole S., Cunha F.Q., Selkirk S., Lorenzetti B.B., Ferreira S.H. Cytokine-mediated inflammatory hyper-algesia limited by interleukin-10. Br. J. Pharmacol. (1995) **115** 684–688.
32 Fiorentino D.F., Bond M.W., Mosmann T.R. Two types of mouse helper T cell IV Th2 clones secrete a factor that inhibits cytokine production by Th1 clones. J. Exp. Med. (1989) **170** 2081–2095.
33 Zlotnik A., Moore K.W. Interleukin-10. Cytokine (1991) **3** 366–371.
34 Howard M. O'Garra A. Biological properties of IL-10. Immunol. Today (1992) **13** 198–200.
35 Bogdan C., Vodovotz Y., Nathan C. Macrophage deactivation by IL-10. J. Exp. Med. (1991) **174** 1549–1555.
36 De Waal Malefyt R., Abrams J., Bennett B., Figdor C.G., Devries J.E. Interleukin-10 (IL-10) inhibits cytokine synthesis by human monocytes: an auto-regulatory role of IL-10 produced by monocytes. J. Exp. Med. (1991) **174** 1209–1220.

37 Fiorentino D.F., Zlotnik A., Mosmann T.R., Howard M., O'Garra A. IL-10 inhibits cytokine production by activated macrophages. J. Immunol. (1991) **147** 3815–3822.
38 Oswald I.P., Wynn T.A., Sher A., James S.L. Interleukin-10 inhibits macrophage microbicidal activity by blocking the endogenous production of tumor necrosis factor α required as a co-stimulatory factor for interferon γ–induced activation. Proc. Natl Acad. Sci. USA (1992) **89** 8676–8680.
39 Chung K.F., Aizawa H., Becker A.B., Frick O., Gold W.M., Nadel J.A. Inhibition of antigen-induced airway hyperresponsiveness by a thromboxane synthetase inhibitor (OKY 046) in allergic dogs. Am. Rev. Respir. Dis. (1986) **134** 258–261.
40 Pavord I.D., Wong C., Williams J., Tattersfield A.E. Effect of inhaled prostaglandin E_2 on allergen-induced asthma. Am. Rev. Respir. Dis. (1993) **148** 87–90.
41 Passargiklian M., Bianco S., Allegra L. Clinical, functional and pathogenetic aspects of bronchial reactivity to prostaglandins $F_{2\alpha}$, E_1 and E_2. Adv. Prostaglandin Thromboxane Res. (1976) **1** 461–475.
42 Passargiklian M., Bianco S., Allegra L. et al. Aspects of bronchial reactivity to prostaglandins, and aspirin in asthmatic patients. Respiration (1977) **34** 79–91.
43 Noveral J.P., Grunstein M.M. Role and mechanism of thromboxane-induced proliferation of cultured airway smooth muscle cells. Am. J. Physiol. (1992) **263** L555–L561.
44 Delamere F., Holland E., Patel S., Bennett J., Pavord I., Knox A. Production of PGE_2 by cultured bovine airways smooth muscle cells and its inhibition by cyclooxygenase inhibitors. Br. J. Pharmacol. (1994) **111** 983–988.
45 Sud'ina G.F., Mirzoeva O.K., Pushkareva M.A., Korshunova G.A., Sumbatyan N.V., Varfolomeev S.D. Caffeic acid phenethyl ester as a lipoxygenase inhibitor with antioxidant properties. FEBS Lett. (1993) **329** 21–24.
46 Toda S., Kumura M., Ohnishi M. Effects of phenolcarboxylic acids on superoxide anion and lipid peroxidation induced by superoxide anion. Planta Med. (1991) **57** 8–10.
47 Baumann J., Von-Bruchhausen F., Wurm G. Flavanoids and related compounds as inhibitors of arachidonic acid peroxidation. Prostaglandins (1980) **20** 627–639.
48 Alcaraz M.J., Hoult J.R.S. Actions of flavonoids and the novel anti-inflammatory flavone, hypolaetin-8-glucoside, on prostaglandin biosynthesis and inactivation. Biochem. Pharmacol. (1985) **34** 2477–2482.
49 Lee T.P., Matteliano M.L., Middleton E. Effect of quercetin on human polymorphonuclear leukocyte lysosomal enzyme release and phospholipid metabolism. Life Sci. (1982) **31** 2765–2774.