I, JORDANA ENIG STOCKHAMER, a Notary Public in and for the Province of Ontario, by Royal Authority duly appointed, residing in the City of Vaughan, in the Regional Municipality of York, in the Province of Ontario, DO HEREBY CERTIFY that the paper writing hereunto annexed, each page of which bears an impression of my seal, is a true and correct copy of A CASE-CONTROL STUDY OF THE EFFECTS OF PHYTOTHERAPY WITH THE ALVEO FORMULA ON IMMUNITY IN CHILDREN signed by Josef Richter, MD, PhD, of the Department of Immunology, Regional Public Health Institute, Usti nad Labem, Czech Republic, the same having been compared by me with the original document, an act whereof being requested I have granted under my hand and notarial seal of office to serve and avail as occasion shall or may require.

DATED at Toronto, Ontario, this 26th day of August, 2010

[Signature]
A Notary Public in and for the Province of Ontario
A case-control study of the effects of phytotherapy with the ALVEO formula on immunity in children

I. Group of children suffering from chronic upper respiratory tract infections and control group.

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Summary:

The increase of herbal medicine use led many scientists to contribute to the research in this field. Also immunologists today recognize the possibility of investigating the scientific value of medicinal products composed essentially of vegetable extracts. Complementary and alternative medicine use more than two-thirds of population. Here we describe the results of some experimental laboratory studies aimed at verifying the efficiency of phytotherapy with the ALVEO formula on immunity in children.

Studies carried out on immunity parameters in saliva – albumin, SlgA in the case group of 27 children undergoing long-term treatment with ALVEO and the control group of 35 children consuming placebo. Every child consumed one bottle of product in an average of 35 days. Clinical evidence of respiratory diseases in children who consumed the ALVEO formula decreased by almost two thirds. We can state that we observed the benefits of regular application of the ALVEO formulation in children improving of salivary immunity and Clinical evidence of respiratory diseases.

Key words: ALVEO, phytotherapy, salivary immunity, respiratory diseases

There is a rising international trend in the application of Complementary and Alternative Medicine (CAM). The World Health Organization (WHO) states that 65-80% of
all health services, treatment and health procedures worldwide are provided by the practices of CAM (2).

The public's growing interest in botanical formulas is notable. There has been a wide application of phytotherapy in the past decade (9, 10, 13, 15, 18, 19, 27, and 29). The general public is becoming more concerned with the use of various herbal formulas, herbal extracts and mixtures. Many clinical case studies based on a wide scale of herbal products have already been published (1, 2, 7, 8, and 19).

This is what inspired us to conduct a study on the effects of the botanical formulation ALVEO. We were mostly interested in the response of clinical changes and the changes in secretory immunity after the administration of Alveo. After studying its individual components we suspected the possibility of positive effects. To observe its impact we selected two groups of children: one group consisted of children being treated at our clinic for chronic upper respiratory tract infections and the other was a control group of healthy children.

ALVEO is a botanical tonic manufactured by Akuna Health Products Inc. in Canada. It was used in the recommended dose of 28 ml per day. The study was conducted between September 2006 and September 2007. Following are the partial results of this study.

**Study methodology:**

The case group (Group A) consisted of 27 children (14 boys and 13 girls) undergoing long-term treatment at the Department of Microbiology and Immunology; the control group (Group B) consisted of 35 children (18 boys and 17 girls) from an elementary school in Usti nad Labem, Czech Republic. The children in Group A were between the ages of 6 and 13 with the average age being 10.95 years; the children in Group B were between the ages of 9 and 14 with the average age being 10.74 years. Group A was given 1 ounce or 28 ml of ALVEO daily on an empty stomach or half an hour after a meal. Every child consumed 1 bottle of product in an average of 35 days. The children in Group B received a placebo which resembled Alveo in appearance and contained the same amount (950 ml) of liquid but no active ingredients. The length of application was averaged 34 days.
The clinical disposition of each child was recorded on a daily basis using a questionnaire with an emphasis on the symptomatology of infections mainly of the upper respiratory tract (cough, phlegm discharge, temperature, rhinitis, pharyngitis, and otitis). Collected data was processed and duration of illness observed. Anthropometric measurements were taken for each child and his/her BMI was calculated.

At the same time, the saliva of each child was collected prior to and after application using the standard method of gathering unstimulated saliva. These specimens were stored in standard plastic test tubes at less than 70 °C until processing. The exact time of collection was observed when the effects of circadian rhythmicity were not present (24). The levels of albumin and IgA in the saliva were determined by immunonephelometry using the Behring and the Dade Behring BN II antisera. The obtained results were grouped according to the following levels: Albumin at 50mg/L of saliva being the norm; 100mg/L being a sign of irritation; 500 mg/L a sign of infection; and amounts greater than 500 mg/L signifying an infection in progress. As for the IgA, normal amounts range between 20 and 150 mg/L of saliva.

The ratio of IgA to albumin was then calculated at all levels in relation to the individual groups observed. The preference of this index being a reduction in the amount of IgA transferred into the saliva as a result of infection.

Data analysis:

Differences in findings for the individual groups observed were compared for each of the groups before and after the application of Alveo and later also in interrelation to the placebo groups and the control files. The average values of the findings were calculated as well as the absolute values and later also the logarithmic values of the mean-root-square errors at 95% CI by means of statistical software such as Microsoft Excel, Instat 3 and Prism 4 (USA). The differences were then evaluated using the Smirnov-Kolmogorov test, the t-test, the ANOVA test, the Tukey-Kramer test.

Ethics:
This case study was conducted in accordance to the Declaration of Helsinki (Fourth revision 1996) and the guidelines for clinical testing in the Czech Republic. Permission was obtained prior to the start of the study from all the parents of the children tested with the formula.

Results:

Table 1 contains the findings before and after the application of the formula and the placebo over the course of 4 weeks (equal to the consumption of one bottle of product). The comparison in the amounts of IgA and albumin in the saliva of the placebo controlled group did not show any significant changes before or after application. On the contrary, the children who were given the ALVEO formulation and who suffered from chronic upper respiratory tract infections showed statistically a highly significant decrease in the amount of IgA and albumin alike.

In studying the occurrences in clinical evidence, we discovered that the clinical symptoms in children who consumed the ALVEO formula decreased by almost two thirds.

Discussion:

Even in the more advanced countries there are both historical and cultural reasons for the use and application of alternative medicine rather than conventional treatments. It is important to acknowledge that for half the world’s population this type of treatment is actually the norm. The use of the term “supplementary”– alternative medicine (complementary – alternative medicine – CAM) stems from the close relationship between the classical and alternative approaches to treatment. This can include alternative physical procedures such as acupuncture and acupressure as well as the application of herbal remedies as practiced by Indian, Japanese and Chinese cultures and in procedures such as phytotherapy and so on (19, 29).

An example would be in the treatment of allergies where a blend of various botanicals was used. The formulation Pollen contains a mixture of 12 different pollens. The reported effect of this product was a decrease in the frequency of allergy symptoms and a lower intake of antibiotics (2). The use of various herbal homeopathic formulas was observed in a series of clinical trials in individuals suffering from allergies, asthma, bronchitis as well as other health conditions (7, 8, 10, 13, 15, 19, 29, and 30). Although the discussion about the positive effects
of homeopathic products on the market remains intense, it is necessary to take a truly qualified stance on the matter; the evidence for which can only be provided by scientific and complex studies (1, 2, and 12). Presently there are no trial results that completely negate the effects of phytotherapeutic products; nor are there any results that entirely support them (2). We discovered that from the total 26 ingredients contained in the tested formula, at least 16 have an effect on the modulation of immunity response while others seem to function via modulation of CNS (10, 30). In present day USA, herbal extracts and the formulas containing herbal extracts are considered to be medicines, food and even dietary supplements. The latter are commonly referred to as DSHEA (4). Some herbal products are considered to be adaptogens - products which have a normalizing (restorative) influence on the body and help defend it against stressors (4, 10). We still require more research about the antioxidant tendencies of herbal extracts, their effects on the regulation of enzymatic functions and their precise influence on the entire immune system (1, 7, and 8). In our observations at the Health Institute we have noticed that herbal extracts have positive effects on the health of children who are already successfully responding to treatment. But even with some of our patients, we realize that short-term application of these herbs (over the course of one month) is not enough to positively influence the observed immunity parameters.

The safety of herbal formulations depends on their composition; though the fact remains that the effects of many herbal extracts are still not known. For example, there are more than 100 different components in tea tree oil alone (29).

Preference should be given to those products, which have undergone a series of laboratory and clinical tests, and most importantly, which have been approved by the appropriate bodies in the respective country. Many widely available folk remedies may actually turn out to be potentially harmful. Therefore, it is important to always notify the public about the possible side-effects and risks involved with taking any particular formula (29). Chamomile can for example trigger an allergic reaction in individuals who have been sensibilized by the allergens of ragweed or chrysanthemum (29). Other herbs such as Garden angelica and rue can highly increase one’s photosensitivity.

Herbs and herbal products demonstrate significant antimicrobial activity. This activity is mainly influenced by the phenolic and polyphenolic substances, which also act as strong antiviral, antibacterial and antifungal agents (7, 13). Terpenoids also demonstrate
antibacterial, antifungal, antiviral and protozoaic activity. Similarly it is with alkaloids, lectins, polypeptides and other substances (7, 13, and 18). Some ingredients in ALVEO such as licorice are important because they induce interferon activity, increase the activity of natural killer (or NK) cells and display antiviral activity including against HIV. Glycyrrhizin has anti-inflammatory and anti-allergic properties (7, 8, 15, and 18). Some ingredients contained in the formulation not only demonstrate significant antimicrobial activity but they also have the ability to modulate the immune system. These traits have been confirmed in a series of clinical trials, mainly in individuals inflicted with respiratory illnesses (2, 20). Additional benefits of the formula are many. There is evidence of favorable impact on skin infections, immune dysfunctions, and fatigue syndrome (29). The use of the formula as a preventative stems from, not only its effects on the modulation of the immune system, but also from its effects against stress and fatigue as well as many its other benefits (21, 22).

In addition to observing the clinical behaviors or reactions of an individual to an ALVEO regime, we also decided to measure the parameters of salivary immunity and IgA, from which we further calculated the ration of Albumin to IgA. The reason for this was to check if our results were correct. We evaluated the diagnoses of the children prior to the study and after its completion. The collection of saliva was carried out in a standard manner and always at the same time of day (23, 25). There is little possibility of comparing the immunological findings after the phytotherapy with those of other studies because of the lack of literature available on this subject. Only isolated studies study the effects of ginseng on the levels of IgA (9, 11). The measuring of IgA in saliva alone is fairly inaccurate because it is way too easily affected by the presence of inflammation or damage to the mucous lining of the oral cavity. This is why we decided to also measure the amounts of albumin in the saliva. The presence of which signifies the presence of an inflammation, irritation or infection (3, 16, 17, 20, 23, 25, 28). We also cannot omit the effects of tonsillectomy on the amounts of both observed components, as in our study on body fat (5, 12). Circadian and seasonal rhythmicity can also significantly influence the findings of certain proteins in saliva (25). This is why it is so important to strictly adhere to a collection schedule. The influence of physical strain on salivary immunity is also worth a mention (5).

In closing, we can state that we observed the benefits of regular application of the ALVEO formulation in children. The conclusions we reached suggest a limitation caused by the short application period. We believe that a course of study over the period of three months
would be optimal. Furthermore, new studies show that the inclusion of new indicators in saliva would be appropriate for the purpose of nutritional immunology (11).

Literature:


Appendix:

Albumin in saliva of children before (1) and after (2) application of Alveo and placebo

![Bar chart showing albumin levels in saliva](chart.png)
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