Immunological Activity of Larch Arabinogalactan and Echinacea: A Preliminary, Randomized, Double-blind, Placebo-controlled Trial

Linda S. Kim, ND, Robert F. Waters, PhD, and Peter M. Burkholder, MD

Abstract
OBJECTIVE: The immunomodulating effects of two Echinacea species, E. purpurea and E. angustifolia and larch arabinogalactan extracted from Larix occidentalis were examined in a randomized, double-blind, placebo-controlled, prospective four-week clinical trial at a naturopathic medical school research center. SUBJECTS/MATERIALS: Forty-eight healthy female volunteers (22-51 y) were randomly assigned to one of six groups: standardized extract of E. purpurea (EP); ultra-refined E. purpurea/E. angustifolia (urEPA); E. purpurea/E. angustifolia (EPA); E. purpurea/E. angustifolia plus larch arabinogalactan (EPALA); larch arabinogalactan (LA); or placebo. METHODS: Immunological tests with enumerative measurements, stool cultures for Lactobacillus acidophilus and yeast, and health-related quality of life (HRQoL) using the Medical Outcomes Study derived SF-36 self-administered questionnaire were assessed at baseline and at four weeks. RESULTS: Complement properdin increased by 21 percent in the EPA group (p<0.05) and by 18 percent in the EPALA group (p<0.05), compared to the placebo group (p>0.05). SF-36 showed improvements in overall physical health, vitality, and emotional health in the same two groups (EPA and EPALA). DISCUSSION: Volunteers in the EPA and EPALA groups had increased production of complement properdin after four weeks of intervention. The increased complement properdin may be an indication of one aspect of immune system stimulation in patients treated with either E. purpurea/E. angustifolia or E. purpurea/E. angustifolia plus larch arabinogalactan.

Introduction
Both Echinacea purpurea and Echinacea angustifolia appear to activate non-specific cellular and humoral immunity and the complement system.1-5 Both of these species stimulate the immune system by increasing the production and activity of leukocytes, lymphocytes, and monocytes, as well as cytokines.6-11 E. purpurea and E. angustifolia have been shown to enhance the immune system in both animal models and clinical

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Original Research

Larch and Echinacea

trials.12-16 The enhanced immune function and phagocytic activities have been documented with natural killer (NK) cells, macrophages, and neutrophils of the reticuloendothelial system. Cytokine productions of gamma-interferon (IFN-γ), tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin-6 (IL-6) are some of the immune modulators released and stimulated by *E. purpurea* and *E. angustifolia*.17-20 In clinical studies, Echinacea reduced symptoms of common cold, influenza, and acute respiratory infections.21-24 Randomized controlled trials demonstrate significant reduction in cold symptoms, increased length of time between infections, and more rapid resolution of viral infections.21-23 There have been studies that did not find statistically significant improvement in treating the common cold with Echinacea.25 In general, however, extensive studies support the immune-stimulating, antitumor, and anti-inflammatory activities of Echinacea.26-30 Echinacea is generally considered to be safe with no significant toxicity or adverse effects.31,32

Constituents of Echinacea include polysaccharides, echinacin, echinacoside, flavonoids, caffeic acid derivatives, essential oils, polyacetylenes, alkylamides, and assorted other chemicals. *E. angustifolia* and *E. purpurea* are the most widely used and extensively studied for their active components in analytical chemistry and clinical application. However, due to the various extraction processes (alcohol, glycerin, standardized extracts, whole plant extracts) and delivery methods (capsule, tablet, tincture, tea) currently available, and the various parts of the Echinacea plant with unknown pharmacodynamics and pharmacokinetics (leaf, stem, flower, root, and their respective biologically active/marker components), it is difficult to determine what form of the botanical is the most effective, safe, and valuable in immune enhancement.33-39

Larch arabinogalactan from *Larix occidentalis* was shown to increase circulating peripheral blood monocytes.40 Tumor cells pretreated with larch arabinogalactan enhanced NK cell cytotoxicity and phagocytic capacities of macrophages and lymphocytes, and increased release of various cytokines, such as IFN-γ, TNF-α, IL-1β, and IL-6.40-45 Larch arabinogalactans are a class of long, densely branched high-molecular weight polysaccharides (10,000-120,000 daltons).46 High-grade arabinogalactan extracted form *Larix occidentalis* is composed of 90-98 percent arabinogalactan, and experimental analysis has determined larch arabinogalactan to be a highly branched molecule of 3,6-beta-D-galactan.46,47 There are numerous patents identified in product development using larch arabinogalactan. According to the Generally Recognized as Safe (GRAS) Notice No. GRN 000047 (FDA, Center for Food Safety & Applied Nutrition, Office of Premarket Approval), functional properties of larch arabinogalactan permit its use as a film-former, foam adhesive, additive, thickener, bulking agent, emulsifier, and as a therapeutic agent. Based on food grade status and numerous studies supporting the safety of larch arabinogalactan, it is considered to be extremely safe with minimum to no toxicity.

Immune-stimulating effects of the combination of Echinacea and larch arabinogalactan have been reported.48 Healthy donor blood treated with a combination of larch arabinogalactan, *E. purpurea*, and *E. angustifolia* in 24-hour incubation showed significant increase in macrophage cell density, and the greatest immune cell stimulation and proliferation when compared to single agent vitamins and minerals. The same study showed the combination Echinacea and larch arabinogalactan had a greater immune-enhancing effect than the individual effects of either Echinacea or larch arabinogalactan alone.

**Objectives**

In this study, the immune-stimulating effects of combination Echinacea and larch arabinogalactan observed in previous *in vitro* studies using healthy donor blood samples were tested in healthy volunteers. A randomized, double-blind, placebo-controlled trial was conducted to address the following: (1) to compare the combination therapy to monotherapies of Echinacea and larch arabinogalactan; (2) to assess immunological outcomes following a treatment period of four weeks; and (3) to assess quality of life outcomes of the
intervention. In this report, the immunomodulating effects of different *E. purpurea* whole herb and *E. angustifolia* root preparations, larch arabinogalactan 90-percent concentration extracted from *Larix occidentalis*, and combination Echinacea and larch arabinogalactan are presented.

**Subjects**

**Subject Population**

The clinical trial was approved by the Institutional Review Board (Human Subject Protection Review Committee) of Southwest College of Naturopathic Medicine & Health Sciences. Subjects were selected according to the study inclusion and exclusion criteria (Table 1). Subjects were recruited with newspaper advertisements and posted announcements. A potential 128 female participants were interviewed in a preliminary screening; of these, 48 were selected to participate and randomly assigned to one of six groups. Only females were included in the study to reduce variability of both outcomes and analysis of results. Subjects with major illness (cancer, diabetes, or cardiovascular or autoimmune/immune diseases), acute illness at enrollment and during study period (upper respiratory tract infections, sinusitis, and other acute infections), or subjects taking any known immune-enhancing or altering supplements or medication were excluded from the study. All subjects were followed at Southwest Naturopathic Medical Center in Scottsdale, Arizona. Each subject provided written, informed consent to participate, and were informed of possible rare transient reactions from taking herbal supplements, such as nausea and stomach discomfort.

**Evaluation of Subjects**

Medical history intake and vital signs were recorded at the beginning of the study. Subjects were interviewed at two weeks to monitor compliance with pill taking and to record any adverse effects or changes in health or daily activities. Lifestyle changes inconsistent with the study requirements during the four-week intervention period were also noted including: excessive alcohol intake, recreational drug or new prescription/non-prescription drug use, strenuous exercise, diet changes, and inclusion of other complementary/alternative therapies that could affect the immune system.

**Materials**

**Intervention Plan**

All subjects took daily doses of the supplement on an empty stomach for four weeks, two capsules in the morning and at bedtime. There were six
groups in the study (Table 2): (1) *E. purpurea* whole herb extract (4% phenols; 1.5 g/day) (EP); (2) *E. purpurea* whole herb extract (4% phenols; 780 mg/day), ultra-refined *E. purpurea* whole herb, and *E. angustifolia* root (680 mg/day) (urEP); (3) *E. purpurea* whole herb extract (4% phenols; 908 mg/day), *E. purpurea* whole herb, and *E. angustifolia* root (680 mg/day) (EPA); (4) *E. purpurea* whole herb extract (4% phenols; 908 mg/day), *E. purpurea* whole herb (464 mg/day), *E. angustifolia* root (36 mg/day) (EPALA); (5) larch arabinogalactan (90%; 1.5 g/day) (LA); or (6) placebo (alfalfa and rice flour; 1.5 g/day). Subjects were compliant and followed study instructions during the four weeks, avoiding new alternative therapies, dietary supplements, and excessive alcohol (Table 3). All capsules were indistinguishable in size, color, and taste. Celestial Seasonings, Inc. and Larex, Inc. provided the investigational supplements and placebo for the study.

### Intervention Analytical Chemistry

The *E. purpurea* whole herb extract (4% phenols), *E. purpurea* whole herb, *E. angustifolia* root, and larch arabinogalactan used in the study were extracted from the same lot numbers. The active components of the Echinacea preparations were analyzed for percent phenols and microbial characteristics by Nutritional Laboratories International (Lolo, MT). Microbial analysis showed the herbal preparations were negative for *E. coli* and *Salmonella*.

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**Table 2. Dose Description**

<table>
<thead>
<tr>
<th>GROUP</th>
<th>INTERVENTION THERAPY</th>
<th>DOSE per day</th>
</tr>
</thead>
<tbody>
<tr>
<td>EP</td>
<td><em>E. purpurea</em> whole herb extract 4% phenols</td>
<td>1500 mg/d</td>
</tr>
<tr>
<td>urEPA</td>
<td><em>E. purpurea</em> whole herb extract 4% phenols</td>
<td>780 mg/d</td>
</tr>
<tr>
<td></td>
<td>Ultra refined <em>E. purpurea</em> whole herb and</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>E. angustifolia</em> root</td>
<td>680 mg/d</td>
</tr>
<tr>
<td>EPA</td>
<td><em>E. purpurea</em> whole herb extract 4% phenols</td>
<td>908 mg/d</td>
</tr>
<tr>
<td></td>
<td><em>E. purpurea</em> whole herb</td>
<td>464 mg/d</td>
</tr>
<tr>
<td></td>
<td><em>E. angustifolia</em> root</td>
<td>36 mg/d</td>
</tr>
<tr>
<td>EPALA</td>
<td><em>E. purpurea</em> whole herb extract 4% phenols</td>
<td>908 mg/d</td>
</tr>
<tr>
<td></td>
<td><em>E. purpurea</em> whole herb</td>
<td>464 mg/d</td>
</tr>
<tr>
<td></td>
<td><em>E. angustifolia</em> root</td>
<td>36 mg/d</td>
</tr>
<tr>
<td></td>
<td>Larch arabinogalactan 90%</td>
<td>1500 mg/d</td>
</tr>
<tr>
<td>LA</td>
<td>Larch arabinogalactan 90%</td>
<td>1500 mg/d</td>
</tr>
<tr>
<td>Placebo</td>
<td>Alfalfa and rice</td>
<td>1500 mg/d</td>
</tr>
</tbody>
</table>
**Methods**

**Criteria for Response**

The effects of the investigational supplements on the subjects’ health and immune function were assessed with immunological tests and subjective reporting on quality of life. Vital signs were measured (blood pressure, radial pulse, respiration rate, and temperature), and blood and stool samples were collected at baseline and at four weeks.

Immunological tests with enumerative measurements included: total white blood cell (WBC), neutrophils, lymphocytes, monocytes; NK cell quantitative; complement properdin (CP); TNF-α; Epstein-Barr Virus viral capsid antigen IgG antibody (EBV VCA IgG Ab); cytomegalovirus IgG antibody (CMV IgG Ab); and aerobic bacterial stool culture for *Lactobacillus acidophilus* and stool fungus culture for yeast in colony forming units per gram (cfu/g). Culture medium selective for gram+ was used for *Lactobacillus acidophilus* and mold-inhibiting medium was used for yeast cultures. The procedure detected colonies with more than 100 organisms per colony (1cfu/g=100 *Lactobacillus acidophilus* or yeast), and colonies of <100 organisms were not reported. ABO blood typing was assessed in all subjects at the beginning of the study. Sonora Quest Laboratory (Phoenix, AZ) and Specialty Laboratory (Santa Monica, CA) performed the enumerative measurements.

Subjective reporting on HRQoL was assessed using the standard SF-36 and Symptoms Specific Assessment (SSA) derived from Medical Outcomes Study (MOS), including gastrointestinal function, sleep pattern, and mood. These instruments were self-administered by subjects at baseline and at four weeks. The SSA was developed by the investigators to specifically address quality of life effects with respect to gastrointestinal function, sleep pattern, and mood. The SF-36 was chosen for its multi-dimensionality, brevity, and previous successful application in a variety of diseases. Reponses to the 36 items on SF-36 assess a number of HRQoL domains, ranging from predominantly social and emotional well-being to overall mental and physical health and vitality.

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**Table 3. Subject Compliance**

<table>
<thead>
<tr>
<th>STUDY REQUIREMENTS</th>
<th>No. (%) OF SUBJECTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>No new physical therapies during study*</td>
<td>48 (100)</td>
</tr>
<tr>
<td>Alcohol &lt; 3 drinks per week during study</td>
<td>48 (100)</td>
</tr>
<tr>
<td>Compliance to dosage and pill-taking 100%</td>
<td>5 (62)</td>
</tr>
<tr>
<td>EP</td>
<td>8 (100)</td>
</tr>
<tr>
<td>urEPA</td>
<td>8 (100)</td>
</tr>
<tr>
<td>EPA</td>
<td>8 (100)</td>
</tr>
<tr>
<td>EPALA</td>
<td>8 (100)</td>
</tr>
<tr>
<td>LA</td>
<td>8 (100)</td>
</tr>
<tr>
<td>Placebo</td>
<td>8 (100)</td>
</tr>
<tr>
<td>No new supplements during study†</td>
<td>8 (100)</td>
</tr>
<tr>
<td>EP</td>
<td>8 (100)</td>
</tr>
<tr>
<td>urEPA</td>
<td>8 (100)</td>
</tr>
<tr>
<td>EPA</td>
<td>8 (100)</td>
</tr>
<tr>
<td>EPALA</td>
<td>8 (100)</td>
</tr>
<tr>
<td>LA</td>
<td>8 (100)</td>
</tr>
<tr>
<td>Placebo</td>
<td>7 (90)</td>
</tr>
</tbody>
</table>

*Physical therapies including acupuncture, chiropractic, and other musculoskeletal/connective tissue therapies.†Supplements including nutritional, herbal, and other complementary/alternative therapies.
Table 4. Mean Complement Properdin and TNF-α and their P values

<table>
<thead>
<tr>
<th>GROUP</th>
<th>COMPLEMENT PROPERDIN</th>
<th>TNF-α</th>
<th>P VALUES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BASELINE %</td>
<td>WEEK 4 %</td>
<td>BASELINE pg/mL</td>
</tr>
<tr>
<td>EP</td>
<td>94 + 25</td>
<td>95 + 33</td>
<td>15 + 23</td>
</tr>
<tr>
<td>urEPA</td>
<td>71 + 30</td>
<td>94 + 24</td>
<td>8 + 2</td>
</tr>
<tr>
<td>EPA</td>
<td>60 + 30</td>
<td>86 + 24</td>
<td>14 + 9</td>
</tr>
<tr>
<td>EPALA</td>
<td>47 + 27</td>
<td>70 + 25</td>
<td>15 + 7</td>
</tr>
<tr>
<td>LA</td>
<td>112 + 97</td>
<td>72 + 29</td>
<td>12 + 8</td>
</tr>
<tr>
<td>Placebo</td>
<td>96 + 29</td>
<td>101 + 34</td>
<td>17 + 32</td>
</tr>
</tbody>
</table>

Plus-minus values are means ±SD. NS denotes no significance. P values by two-tailed Student’s t-test.

Statistical Analysis

The two-tailed Student’s t-test was used to assess the differences between baseline and end of treatment. The outcomes of the five active groups and the placebo group were compared between baseline and four weeks. Self-administered SF-36 and SSA were also statistically analyzed for significance in the study groups.

Figure 1. Mean Complement Properdin
**Results**

**Lab Parameters**

Complement properdin percent concentration increased significantly in the EPA and EPALA groups (Table 4). At four weeks the CP in the EPA group was (mean [±SD]) 86±24 (baseline 60±30) and in EPALA was 70±25 (baseline 47±27), compared with 101±34 (baseline 96±29) in the placebo group (Figure 1). CP difference in the EPA group between baseline and four weeks was 26±27 (21.0% increase) (p=0.029) and in the EPALA group it was 22±19 (18.0% increase) (p=0.020); while in the placebo group it was 5±34 (p=0.687). TNF-α decreased in urEPA, EPALA, and LA groups (p=0.040, p=0.034, and p=0.044, respectively) (Table 4). There were no statistically significant pattern changes in the hematological or other immunological serum chemistry (Table 5). Aerobic bacterial culture for *Lactobacillus acidophilus* and fungus culture for yeast showed no significant results.

**Quality of Life Assessment**

SF-36 improved in the EPA and EPALA groups at end of treatment (p=0.042 and p=0.031, respectively) (Table 6), and showed enhanced vitality and physical, emotional and mental health. The SSA decreased in the EPA and EPALA groups (p=0.003 and p=0.015, respectively) (Table 6). The decreases in SSA mean scores demonstrate improvement in gastrointestinal function, sleep pattern, and mood (Figure 2).

Subjects also reported increased bowel movements (BM) and changes in stool characteristic (looser quality and larger quantity) in the following frequency: EP, 4 of 8 subjects (50%); urEPA, 3 of 7 (43%); EPA, 4 of 8 (50%); EPALA, 3 of 7 (43%); LA, 6 of 8 (75%); and placebo, 2 of 8 (25%). The changes in BM reports were most dramatic in the larch arabinogalactan group, particularly in the quality of stool consistency. The majority of subjects in this group experienced varying degrees of changes in their BM habits. None of the subjects reported any discomfort and did not discontinue the study due to BM changes.
Adverse Reactions

Two of the 48 subjects experienced adverse reactions two weeks into the study. One volunteer in the urEPA group discontinued due to self-reported anxiety, nervousness, and heart palpitation while taking the supplements. Another volunteer in the EP group reported bilateral arthritic symptoms over her wrist, metacarpophalangeal, and proximal interphalangeal joints; however, the symptoms were similar in location and quality to arthritic symptoms experienced over 10 years previously. The symptoms of the two subjects resolved without complication upon discontinuing the supplement.

Discussion

*E. purpurea*, *E. angustifolia*, and larch arabinogalactan supplements were generally well tolerated. Gastrointestinal function, sleep pattern, mood, and emotional health items of SSA improved.

Table 6. P values of SF-36 and Symptoms Specific Assessment

<table>
<thead>
<tr>
<th>GROUP</th>
<th>SF-36</th>
<th>Symptoms Specific Assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>EP</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>urEPA</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>EPA</td>
<td>0.042</td>
<td>0.003</td>
</tr>
<tr>
<td>EPALA</td>
<td>0.031</td>
<td>0.015</td>
</tr>
<tr>
<td>LA</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Placebo</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS denotes no significance. P values by two-tailed Student’s t-test.

Figure 2. Mean Scores of Symptoms Specific Assessment
significantly in the same groups with higher SF-36 scores, which were the EPA and EPALA groups. Based on SF-36 and SSA outcomes related to HRQoL, the most significant benefits to subjects’ health and vitality were observed in the EPA and EPALA groups.

The dense polysaccharides of larch arabinogalactan are considered a good source of dietary fiber, improving intestinal microflora such as *Bifidobacterium*, *Lactobacillus acidophilus*, and *Enterobacteriacea* in human studies.53-55 Larch arabinogalactan fibers are fermented by gastrointestinal microflora resulting in the production of short-chain fatty acids (especially butyrate and propionate). These fatty acids are of particular value to colonocytes, and are the intestinal epithelial cells’ preferred fuel for energy generation. The fiber dense quality of larch arabinogalactan may explain the greater changes in BM from the LA intervention than from the Echinacea formulas or placebo. Although many of the subjects had changes in their digestive habits, no conclusive data pertaining to stool cultures of *Lactobacillus acidophilus* and yeast emerged from the study. Vital signs collected at baseline and four weeks showed no negative or positive correlation with the treatment intervention. No statistical correlations or patterns were observed with blood type and outcomes of the study; the randomization of subjects to the six groups was not matched for blood types at the beginning of the study.

The increase in complement properdin concentrations in the *E. purpurea/E. angustifolia* and *E. purpurea/E. angustifolia* plus larch arabinogalactan groups may indicate stimulation of the complement immune system. Biological activities associated with complement activation include cell lysis, opsonization, enhanced phagocytosis, complement receptor activation, chemotaxis, activation of neutrophils and monocytes, and clearance of immune complexes.56,57 The stimulation by EPA and EPALA may result in enhanced phagocytosis and immune function, and increased production and release of cytokines, which can induce expression of other cytokines and immune cells, further benefiting the specific and non-specific immune system. However, due to the large variance in the baseline values of the six groups, the outcomes of the study should be considered cautiously in supporting immune effects of the interventions. Thus, TNF-α decreases (p<0.05) in the three groups – urEPA, EPALA, and LA – and complement properdin changes (increased in EPA and EPALA and decreased in LA) (Table 4) do not suggest immunomodulating activities of the intervention in this study and remain suspect, requiring further investigation.

The preliminary nature of the project did not include extensive screening of the subjects prior to enrollment. Testing their WBC and other immune parameters with requisite ranges (upper and lower limits) and examination of their medical records with focused subject selection criteria may have prevented such high variance in the baseline values. The wide age range (22-51); personal/professional background (student, working professional, etc.); diverse stress indicators, diet behaviors, and daily activities of the subjects; and lack of matching based on such diversity may have contributed to the dissimilar lab values. Lack of statistically significant activities of Echinacea and larch arabinogalactan formulas in this study may also be the result of methodology used – subject characteristics, insufficient sample size, and inadequate length of therapy.

This study raises questions leading to future proposals to further explore the immune effects of Echinacea and larch arabinogalactan. Previous clinical trials have shown increased secretion and activity of leukocytes and cytokines (IFN-γ, TNF-α, IL-1β, and IL-6), while other studies have supported the benefits of Echinacea and larch arabinogalactan in reducing symptoms and recovery time from acute respiratory tract infections, such as the common cold and influenza. Activation of the complement system and increased production of complement properdin may be another immune factor stimulated by Echinacea and combination Echinacea and larch arabinogalactan. Demonstrating clinical efficacy and pharmacodynamics of Echinacea and larch arabinogalactan require more research to understand the components involved in stimulating the natural immune defense system.
In addition to evaluating the levels of complement properdin, measurement of other components of the complement system may provide more conclusive evidence on complement system activation by Echinacea and larch arabinogalactan. The role of Echinacea and larch arabinogalactan to stimulate the specific and non-specific immune system should also be further explored to understand the specific antimicrobial and anti-inflammatory actions of these immunomodulators. Future studies designed to assess immune response to both short- and long-term interventions and antigen-induced immune responses may provide better understanding of these botanical extracts.

Acknowledgments

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