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The Effect of *Echinacea purpurea* on Stimulating IgM (Primary) and IgG (Secondary) Immune Responses in Male CD1 Mice

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ABSTRACT

Echinacea purpurea, commonly referred to as the purple coneflower, is native to the United States and was first discovered by Native American Indians as a medicinal herb. Liquid extracts of Echinacea purpurea were evaluated for the capacity to stimulate total immunoglobulin immune responses in male CD1 (Charles River Laboratory) mice infected with an antigen of SRBC (sheep red blood cells). A control group (n=14) received the antigen but no Echinacea and a treatment group (n=15) received both the antigen and the Echinacea extract. Blood samples, obtained by cardiac puncture, were taken 48 hours after the second and final antigen injection. Blood samples were purified for IgG antibodies by the isolation of the IgG fraction (containing antibodies of all specifities) in a complex mixture by precipitation with saturated ammonium sulfate (SAS). In a 10% buffer of PBS, samples were then dialyzed for a 48 hour period at 4°C against three buffer changes (10% PBS). Once completing dialysis, samples were placed in the mass spectrophotometer for direct UV measurement of protein present in each sample. Protein concentration in samples revealed no significant difference between control and treatment groups. These results imply that Echinacea purpurea has no effect on the total number of antibodies, and IgG, or a B-cell mediated response, was never activated.

Keywords: *Echinacea purpurea*

INTRODUCTION

"Echinacea, the Purple Coneflower, is indigenous to the plains of the Untied States and was first discovered as an immune stimulant by Native American tribes around the seventeenth century" (Hobbs 1990). Plains Indians revered Echinacea for alleviating toothaches, sore throats, coughs, infections, snakebites and numerous other diseases and afflictions. But Echinacea did not become renowned until the advent of H.C.F. Meyer, a German physician from Nebraska. Around 1870, Meyer formulated and began selling a patent medicine containing Echinacea that became popular. The late eighteenth century revealed the first article on Echinacea in a medical journal. Soon after, Echinacea was adopted for use by homeopathic doctors and recognized as an immune stimulant that increases the attack of white blood cells on bacteria and waste material. Between 1930 and 1980, more than four hundred scientific journal articles appeared exploring the medicinal properties of Echinacea. Around 1986, U.S. herbalists were introduced to Echinacea, leading to it becoming the most popular herb in medicinal practice to date.

"One of the most prevalent uses of Echinacea is to forestall or shorten the common cold through increasing the immune systems phagocytic function, white blood cell and macrophage count" (Weil 1995). The concept of increasing the components that make up the immune system presents a healthier and less destructive means to ward off certain types of infections than traditional western medicinal therapies. For example, today's society has become dependent on treatments of antibiotics as the "cure-all" for minor ailments which has contributed to three problems. First, the effectiveness of treatments of antibiotics to fight infections after prolonged use has come under question. Meaning, after prolonged use of an antibiotic an individual builds up immunity towards the drug yielding a reduced ability of the drug to ward off an infection.

This leads to the second problem with traditional treatments of antibiotics. Prolonged use of an antibiotic can result in the evolution of resistant microorganisms. Antibiotic resistant organisms, coupled with the over prescribing of antibiotics for common ailments, place a tremendous strain on physicians and researchers to eliminate more serious infections. The invention of more powerful and successful antibiotics is being surpassed by the evolution of resistant microorganisms. This concept is already resulting in diseases from our past coming back to haunt us, so to speak. An appropriate illustration of this problem can be observed on the East Coast of the United States where researchers are discovering antibiotic resistant forms of tuberculosis.

The third, and most concerning, problem with treating common ailments by way of antibiotics is what they do to the immune system on a cellular level. When antibiotics first emerged, they were tremendously effective at killing infections. However, over time organisms have become resistant to each generation of antibiotics, requiring the creation of stronger drugs to kill the now resistant organisms from previous treatments. This cycle has repeated up until present times. Today's antibiotics are manufactured so strong that they have been termed "loaded guns" by health professionals. These groups of antibiotics not only kill bacterial cells, but they also indiscriminately destroy healthy cells, which constitute the "fighters" (Luettig, et al, 1989) of infections in our immune system. Instead of actually benefiting the human body,

an individual's immune system becomes suppressed as a result of this indiscriminatory cell destruction caused by treatments of antibiotics. Once the immune system is compromised, an individual is vulnerable to other infections.

The relevance of Echinacea purpurea as a non-specific immune stimulant is to accomplish similar objectives as antibiotics but in a healthier and less destructive manor, while providing numerous benefits to the body beyond the realm of antibiotics.

MATERIALS AND METHODS

In this experiment, CD1 (Charles River Laboratory) mice were allowed to acclimate three per cage for three weeks prior to the beginning of the study. 140μL of Echinacea extract was diluted in 4L of distilled-deionized water and dispensed to the treatment group (T1) in their water bottles proceeding acclimatization. The control group (C1) received distilled-deionized water. The administration of the Echinacea extract remained the only treatment for two weeks.

After two weeks, the antigen of sheep RBC's (1% SRBC and PBS, pH=7.2) was injected subcutaneously in the dorsal region of each subject in both treatment (T1) and control (C1) groups. The initial antigen injection was allotted one week to theoretically produce the intended primary, or IgM, immune response. Directly proceeding that week, the same concentration and volume of antigen was readministered similarly to all subjects in order to produce the secondary, or IgG, immune response.

One week following the secondary antigen injection, subjects were gathered for a blood sample by cardiac puncture. Subjects were anesthetized with a peritoneal injection of Ketamine (.1mL Ketaset/10g mouse body weight), and blood was withdrawn through a B-D 25-gauge needle with a 5mL syringe inserted into the heart. Mice were then sacrificed by decapitation.

Blood samples were purified through the isolation of the IgG fraction (containing antibodies of all specifities) in a complex mixture by precipitation with saturated ammonium sulfate (SAS) (33% SAS solution+67% PBS, pH=7.2). The IgG containing precipitate was dissolved in a cold buffer of PBS (10% of original volume) by gentle vortexing. Samples containing the IgG solution were each placed in dialysis membrane and sealed, then dialyzed over 48 hours at 4°C against three changes of 2000mL PBS (buffer).

Once dialysis was complete, 200μL of the original volume was pipetted from each sample and diluted with 800μL of PBS (bringing the total volume of sample to 1000μL) in labeled 1.5mL test tubes. This solution was then transferred to spectrophotometer test tubes for protein analysis. Samples were analyzed using the mass spectrophotometer for direct UV measurement of the protein concentration at a wavelength of 280nm.

RESULTS

The overall immune response to sheep red blood cells displayed no significant difference in the protein concentration between the treatment (T1) and control (C1) groups. After adding both the mean and standard deviation of the protein concentration results, only a 14% difference was observed between the two experimental groups. The difference in protein concentrations, or antibody count, is so slight that the statistical analysis indicates that the means of both groups actually overlap.

Table 1. Protein concentration per individual in the treatment group (T1) after direct UV measurement of protein through spectrophotometry.

ID	Protein
A1	.185 mg/mL
A2	.24 mg/mL
A3	.377mg/mL
B1	.388 mg/mL
B2	.155 mg/mL
B3	.211 mg/mL
C1	.219 mg/mL
C2	.172 mg/mL
C3	.187 mg/mL
D1	.186 mg/mL
D2	.169 mg/mL
D3	.227 mg/mL
E1	.239 mg/mL
E2	.257 mg/mL
E3	.08 mg/mL

Table 2. Protein concentration per individual in the control group (C1) after direct UV measurement of protein through spectrophotometry.

ID	Protein
A1	.124 mg/mL
A2	.25 mg/mL
A3	.188 mg/mL
B1	.187 mg/mL
B2	.303 mg/mL
B3	.151 mg/mL
C1	.17 mg/mL
C2	.146 mg/mL
C3	.281 mg/mL
D1	.198 mg/mL
D2	.333 mg/mL
D3	.275 mg/mL
E2	.666 mg/mL
E3	.299 mg/mL

DISCUSSION

Results obtained in this experiment display very little variance in the protein concentration, or antibody count, between the treatment (T1) and control (C1) groups. After dividing the sum of the means and standard deviations {(T1) .2436/(C1) .02188=14%} of the two experimental groups, only a 14% difference in the total antibody count exists between the study groups.

Therefore, results derived in this experiment indicate that Echinacea purpurea extract has no effect on the total number of antibodies produced. Furthermore, the evidence in this experiment seems to indicate that IgG, or a B-cell mediated response, was never activated in response to SRBC's (sheep red blood cells).

Upon interpretation of the data, two possible rationales were discovered that may very well have contributed to the results in this study. First, it was contrived that the antigen of SRBC's (sheep red blood cells) tends to activate a T-cell response instead of an antibody, or B-cell mediated response (Sambrook, et al, 1989). If this experiment was to be repeated, the antigen of SRBC's would be replaced with "Fruend's Complete Adjuvant," and antigen that produces more antibodies through increasing immune responses to particular or specific proteins (Sambrook, et al, 1989). The second exposition of the data yielded in this study reveals evidence of possible contaminated blood samples after the IgG purification procedure. Samples should have appeared clear and transparent upon purification with saturated ammonium sulfate (SAS). However, a few samples were viewed to contain a reddish tint. The red color, detected post-purification, seems to support the idea of contamination. Based on this, the direct UV measurement of the samples protein concentration may have been masked by other proteins present in the red contaminant. Therefore, the proteins analyzed during mass spectrophotometry may not have been all antibodies but less specific proteins instead.

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