

The Effects of Ginseng and Echinacea on Some Plasma Cytokine Levels in Rats ^[1]

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Summary

In this study, it was aimed to determine the effects of ginseng and echinacea on plasma levels of IL-10, TGF- β 1, TNF- α in healthy rats. In this research, 48 male Fischer 344 rats were divided into three equal groups as Control (K), Ginseng (G) and Echinacea (E). The control group were fed the standart rat diet (Purina®) as ad libitum for 40 days, whereas G and E groups animals received the same diet containing 0.5 g/kg *Panax ginseng* and 0.75 g/kg *Echinacea purpurea*, respectively. At 20th and 40th days, plasma levels of the IL-10, TGF- β 1, TNF- α were assessed. At the 20th day, plasma IL-10 level in the ginseng group was higher ($P<0.05$) compared to the control and the echinacea group. There was no difference among the sampling times and the groups in concern with TGF- β 1 level. At the 20th day, TNF- α level was higher ($P<0.05$) in the echinacea group than those of the ginseng and the control group. At the 40th day, TNF- α level in the echinacea group was higher ($P<0.05$) than the control group. In this study, the increases in IL-10 level with ginseng application and in TNF- α level in the echinacea group have supported the view of these plants modulate the immune system. However, it requires to make more detailed study about the effects of these plants on these cytokines.

Keywords: Ginseng, Echinacea, Cytokine, Rat

Ginseng ve Ekinezyanın Ratlarda Bazı Plazma Sitokin Düzeyleri Üzerine Etkileri

Özet

Bu çalışmada sağlıklı ratlarda ginseng ve ekinezyanın IL-10, TGF- β 1, TNF- α 'nın plazma seviyeleri üzerine etkilerinin belirlenmesi amaçlanmıştır. Araştırmada 48 erkek Fischer 344 sağlıklı rat kullanıldı. Hayvanlar Kontrol (K), Ginseng (G) ve Ekinezya (E) olmak üzere üç eşit gruba ayrıldı. Kontrol grubundaki hayvanlar 40 gün süre ile standart rat yemi (Purina®) ile ad libitum olarak beslenirken, G grubundaki hayvanlara 0.5 g/kg yem olacak şekilde *Panax ginseng*, E grubundaki hayvanlara ise 0.75 g/kg yem olacak şekilde Ekinezya içeren aynı yem verildi. 20. ve 40. gün sonunda IL-10, TGF- β 1 ve TNF- α plazma düzeyleri belirlendi. Çalışmada 20. gün belirlenen plazma IL-10 düzeyi ginseng grubunda kontrol ve ekinezya grubuna göre yüksekti ($P<0.05$). Çalışmada ginseng ve ekinezya uygulamasına bağlı olarak TGF- β 1'in plazma değerleri açısından gruplar ve örnekleme zamanları arasında herhangi bir farklılık görülmedi. Çalışmada 20. gün ekinezya grubunda belirlenen plazma TNF- α düzeyinin kontrol ve ginseng grubuna göre yüksek olduğu ($P<0.05$) görüldü. Çalışmanın 40. gününde ekinezya grubuna ait plazma TNF- α düzeyinin kontrole göre yüksek olduğu ($P<0.05$) belirlendi. Çalışmada ginseng uygulaması ile plazma IL-10 düzeyindeki önemli artışlar ile ekinezya grubunda belirlenen plazma TNF- α değerindeki önemli farklılıklar bu bitkilerin immun sistemi modüle ettiği yönündeki görüşleri destekler niteliktedir. Sonuç olarak, uygulanan bitkilerin bu sitokinler üzerine etkilerine dair daha detaylı çalışmaların yapılması gerekmektedir.

Anahtar sözcükler: Ginseng, Ekinezya, Sitokin, Rat

INTRODUCTION

Since many years, the roots or leaves of several varieties of *Panax* plants (e.g. *Panax ginseng*, *Panax notoginseng*,

Panax japonicus and *Panax quinquefolium*) have been used in many disorders, such as inflammation, cardiovascular



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diseases, wound healing, sexual function and cancer as well as for enhancing physical strength^{1,2}. Bioactive compounds of ginseng are glycosidal saponins known as ginsenosides³.

There are three commonly grown species of echinacea: *Echinacea purpurea*, *Echinacea angustifolia*, and *Echinacea pallida*. Echinacea is marketed as tablet, capsule, and liquid in the United States. In modern day, echinacea became popular plant with the belief of its immune stimulant effects on upper respiratory infections (URI), fevers, urinary tract infections, chronic cough. It has also been promoted as a general immune stimulant to help fight various other infections. Topical preparations are also available for treatment of wounds and inflammatory skin conditions⁴.

Cytokines are secreted proteins that influence the survival, proliferation, differentiation and functional activity of cells of the immune system, as well as of most other organ systems⁵. Interleukin-10 is a multifunctional cytokine and an anti-inflammatory, which regulates the function of various cell types of the immune system. IL-10 as a product of Th2 subset of CD4 T cells suppressed the lymphokine production by Th1 cells⁶. Its principal function seems to be containment and eventual termination of inflammatory responses, thus IL-10 facilitates elimination of infectious organisms with minimal damage to host tissues. In addition, IL-10 plays important roles in immune tolerance, T cell and DC (Dendritic cell) development, and growth and differentiation of B cells⁷.

The other Th2 cytokine Transforming Growth Factor β (TGF- β) is a multifunctional polypeptide hormone which regulates a variety of important cell and tissue functions, such as cell growth and differentiation, proliferation, chemotaxis, apoptosis, angiogenesis, immune responses, extracellular matrix production, and hematopoiesis⁸. TGF- β also has diverse effects on a variety of cell types to regulate many complex multicellular systems⁹. The complexity and diversity of TGF- β 's function is demonstrated through its multiple roles in immune system suppression, wound healing, fibrosis, development, and oncogenesis¹⁰.

Tumor necrosis factor- α (TNF- α) is a multifunctional and pro-inflammatory cytokine. TNF- α is produced by cells of the monocyte series, and possibly natural killer cells, that exerts a cytotoxic or cytostatic activity on some tumor cell lines¹¹. It has been shown that TNF- α can selectively lyse certain transformed cells. On the other hand, it mediates severe inflammatory reactions through regulating T or B cell responses and stimulating several cytokines from different immun cell lines and inducing class I and class II MHC molecules. Thus, TNF- α is a potent immunoregulatory molecule¹².

In recent years, there have been interest in ginseng, echinacea and their immunomodulator effects including cytokines. It has been reported that ginseng administration

increased some anti-inflammatory cytokines such as IL-10 and TGF- β 1 levels^{13,14}, whereas some authors have obtained decreases in the same parameters^{15,16}. On the other hand, IL-10 and TGF- β 1 levels decreased with echinacea application^{17,18} and vice versa¹⁹. However, there are different data related with TNF- α levels in ginseng and echinacea researches. Pannaci²⁰ determined that ginseng administration increased TNF- α levels, while in the other study¹⁶ it has been observed decreases in the same cytokine level. Some reports indicate that echinacea as a natural immunostimulant increased TNF- α levels²¹, although there are contrary findings²².

Thus, it was the aim of this study to investigate the effects of ginseng and echinacea powders on plasma levels of both pro-inflammatory cytokine TNF- α and anti-inflammatory cytokines IL-10 and TGF- β 1 in rats.

MATERIAL and METHODS

In this study, 48 male, healthy Fischer 344 rats were used. The rats were divided into 3 equal groups where the weight of each group of animals was close to each other. All the rats were kept in individual cages during the experiment (40 days) and were fed ad libitum as follows: Group 1 (K) fed with pellet food, Group 2 (G) fed with pellet food containing 0.5 g/kg Panax Ginseng root powder, Group 3 (E) fed with pellet food containing 0.75 g/kg *Echinacea purpurea* root powder. At the 20th and 40th days of study, citrated blood samples were taken from randomly 8 animals of each group. In these blood samples, IL-10, TGF- β 1 and TNF- α plasma levels were determined with ELISA (Bio-Tek Instruments, Inc) using sandwich enzyme-linked immunosorbent method via commercial kits (Biosource). Ethical Committee of the Faculty of Veterinary Medicine (Report No: 2007/036) approved the study protocol.

Statistical differences between sampling times and among the groups were tested by student's t-test and Duncan's multiple range test, respectively²³.

RESULT

At the 20th day of the study, plasma IL-10 levels in the ginseng group was found to be higher ($P < 0.05$) when compared to the control and the echinacea groups (*Table 1*). At the 40th day, there were no differences among the groups and the sampling times in regarding to IL-10 (*Table 1*). Depending on applications of ginseng and echinacea, there were no differences for TGF- β 1 plasma levels among the sampling times and the groups (*Table 2*). At the 20th day, the level of plasma TNF- α determined in the echinacea group was higher ($P < 0.05$) compared to that of the ginseng and the control group levels (*Table 3*). At the 40th day of study, the level of TNF- α plasma in the echinacea group was just different ($P < 0.05$) from the control group (*Table 3*).

Table 1. Effects of ginseng and echinacea on the plasma IL-10 levels in rats (means±SEM, n=8) (pg/ml)

Tablo 1. Ginseng ve ekinezyanın ratlarda plazma IL-10 düzeyleri üzerine etkileri (means±SEM, n=8) (pg/ml)

Groups	20th Day	40th Day
Control	37.72±0.94b	38.91±1.33
Echinacea	35.77±1.39b	36.63±1.58
Ginseng	41.19±0.88a	40.59±1.17

a, b: Different letters in the same column refers the differences among the groups (P<0.05)

Table 2. Effects of ginseng and echinacea on the plasma TGF-β1 levels in rats (means±SEM, n=8) (pg/ml)

Tablo 2. Ginseng ve ekinezyanın ratlarda plazma TGF-β1 düzeyleri üzerine etkileri (means±SEM, n=8) (pg/ml)

Groups	20th Day	40th Day
Control	105.58±1.70	105.07±2.82
Echinacea	104.82±1.75	104.94±2.61
Ginseng	105.07±2.07	107.73±2.92

Table 3. Effects of ginseng and echinacea on the plasma TNF-α levels in rats (means±SEM, n=8) (pg/ml)

Tablo 3. Ginseng ve ekinezyanın ratlarda plazma TNF-α düzeyleri üzerine etkileri (means±SEM, n=8) (pg/ml)

Groups	20th Day	40th Day
Control	23.56±1.04b	23.79±1.03b
Echinacea	29.75±0.65a	27.61±1.20a
Ginseng	25.75±0.88b	25.91±0.99ab

a, b: Different letters in the same column refers the differences among the groups (P<0.05)

DISCUSSION

In paralel with our results, Yang¹⁴ noted that ginsenoside Rd increased IL-10 expression levels in mice. Further Huang²⁴ reported that ginseng extract administration caused the increase IL-10 levels in human lymphocyte cell culture. In addition, Rivera²⁵ also reported increase plasma levels of IL-10 in mice infected with parvovirus. In contrary to above reports, Ahn¹⁶ suggested that ginseng polysaccharide decreased the levels of IL-10 in mice. It has been reported that ginseng extract caused no changes in polymorph nuclear leukocytes culture²⁶. Balance between pro- and anti-inflammatory cytokines in many inflammation and infections is important for host immun defence thus the increase in IL-10 with ginseng administration is accepted beneficial in immunomodulation²⁷. In this study, we found that Echinacea administration unchanged IL-10 level in accordance with the reports of Zhai²⁸. Senchina¹⁸ reported that echinacea extract administration suppressed the production of IL-10 in human blood cell culture. On the other hand, there are various studies in related to the increase in IL-10 with the echinacea extracts^{19,29}. It is expressed that increase in IL-10 levels as a result of

echinacea administration may dampen over-activated inflammatory responses³⁰.

In this study, TGF-β1 levels showed no significant changes in ginseng and echinacea groups. However, there are controversy over the effects of these plants on TGF-β1 levels. Kanzaki¹³ reported that ginseng saponin increases TGF-β1 levels in human skin fibroblast cultures. In contrast, Lee³¹ noted that ginseng extract administration decreases TGF-β production in human lymphocyte cell cultures treated with LPS. Similarly, Chen¹⁵ determined a decline in TGF-β1 levels in mice fed a mixture plants containing ginseng. Randolph¹⁷ reported that echinacea extracts decreased TGF-β mRNA expression in human Th1 cell culture. Release of the immunosuppressive cytokine TGF-β is regulated by CB2 receptors in peripheral blood lymphocytes. Stimulating of CB2 receptors in blood lymphocyte increases TGF-β production. TGF-β is known to play an important role in the regulation of the other cytokines such as TNF-α and IL-1³². Although alkylamides in echinacea is a CB2 receptor stimulant and affects cytokine production³³, lack of effect of ginseng and echinacea on TGF-β1 levels in this study may be due to used healthy material, herbs amounts, application route and the interaction with the other cytokines.

In paralelly to our results, Shin³⁴ noted that oral administrated ginseng extract do not cause any changes TNF-α levels in mice. On the other hand, Yang¹⁴ noted that ginsenoside Rd administration increased TNF-α levels in rats. Similarly, Pannacci²⁰ reported that oral administrated ginseng extract elevated TNF-α levels in peritoneal cell cultures from mice. In contrary, Wang³⁵ suggested that ginseng saponin suppressed the increases in levels of TNF-α in scald mice peritoneal macrophage cultures. Ahn¹⁶ also suggested that ginseng administration decreases TNF-α levels in *S. aureus*-infected mice. No changes in TNF-α levels with the ginseng administration is comprehensible when different and also contrary results are considered together. Although there are contrary results²², in the present study, the increases in TNF-α levels as a result of supplementation of the diet with echinacea are in agreement with the results of previous studies in which the elevations in TNF-α levels were reported in cell cultures with echinacea extracts^{21,18}. In the other study, Rininger¹⁹ noted that echinacea purpurea increased TNF-α levels in mice. Similarly to above results, it has been suggested that echinacea extract increased TNF-α levels in cell culture treated with LPS²⁸.

The potent modulatory action of echinacea alkylamides on TNF-α expression was shown in human monocytes. It is explained that this effect is mediated via the cannabinoid receptor CB2³⁶. Based on this data, it has been claimed that echinacea-induced stimulation of immune cells such as macrophages to produce TNF-α, IL-1, IL-6, and NO could serve to augment the immune response and more rapidly attenuate cold and flu symptoms¹⁹.

It appears likely that echinacea and ginseng activates the immune system via cytokine pathways through as yet unknown mechanisms. Further studies are needed to arrive at a definitive opinion about the effects of these herbs on cytokines complexity.

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