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Research Article

Effects of Long Term Administration of *Echinacea* in Addition with Vaccination on General Condition and Liver and Renal Performance in Dogs

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ABSTRACT

Echinacea is one of the more recognized herbs associated with immune modulation in human and animals. Until now little work has been done to evaluate its effects in dogs. The aim of this study was to evaluate the effects of long-term administration of *Echinacea* on some biochemical and hematological parameters in dogs. For this purpose, 15 adult healthy dogs of mixed breed were divided into three equal groups. Dogs in group 1 received orally extract of *Echinacea purpura* for a period of 28 days and canine polyvalent vaccine on the seventh day.Dogs in groups 2 and 3 received *Echinacea purpura* and canine polyvalent vaccine, respectively, at values similar to those of group 1.On days 0, 28 and 56 days after Echinacea feeding, blood samples were collected from all dogs and serum hematologic and biochemical parameters including alanine aminotransferase, aspartate aminotransferase, blood urea nitrogen, creatinine, albumin and total protein were measured.Echinacea increased significantly the number of red and white blood cells as well as lymphocytes and hemoglobin and also significantly decreased the number of neutrophils ($p<0.05$). Vaccination alone resulted in a significant increase in white blood cell count ($p<0.05$). This study proved that long-term feeding of Echinacea in dogs while increased blood parameters, especially the red blood cells, had no complications and toxicity on hematologic and biochemical parameters.

Keywords: Echinacea, Vaccination, Hematology, Biochemistry, Dog.

INTRODUCTION

Today, the effects of medicinal plants on the immune system have been studied by many researchers (Sharma 2010). Echinacea is one of the more recognized herbs associated with immune modulation (Thacker 2010). The active components which occur in the various Echinacea preparations can be divided into three major groups: caffeic acid derivatives, polysaccharides, and lipophilic components (Bauer and Wagner 1991). In humans, it has been reported to impact the innate immune system by increasing the activity of phagocytic cells, promoting production of various cytokines, and enhancing the activity of natural killer cells (Sullivan et al. 2008). Fluid extracts of *Echinacea purpurea* are widely used for the prevention and treatment of colds and respiratory infections (Grimm and Müller 1999). It is concluded that Echinacea extract has prophylactic effect on incidence of phenytoin-induced cleft palate in mice (KhaksaryMahabady et al. 2006). Also, O`Neill et al. (2002) was concluded that Echinacea effectively stimulates equine immunocompetence, and the plant extract behaves, in equine subjects, as a hematinic agent, i.e. one which improves the quality of blood by increasing hemoglobin levels and the number of erythrocytes. The extract of Echinacea purpurea can support the immune system by increasing the number of white blood cells and can be effective in hematopoiesis by increasing the number of red blood cells in mice (Modaresi 2013). In addition, Oskoii et al. (2012) suggested that *Echinacea purpurea* administration exerted positive effects on growth and biochemical and hematological indices in rainbow trout. The addition of Echinacea to the human T cell culture has led to significantly depression of IL-2 production but not cell viability and significantly enhanced phagocytic activity of alveolar macrophages (Sasagawa et al. 2006).A decline in T-cell-mediated immunity and transient state of immunosuppression after immunization has been reported in dogs (McDonald 1992, Miyamoto et al. 1995, Foley et al.

1999). Sometimes the vaccination fails, or leads to the development of latent diseases (Strasser et al. 2003). On the other hand, it is believed that the use of immunomodulators increases the effectiveness of the vaccine (Barrett 2003). So that in a study, Echinacea purpura the efficacy of the influenza vaccine has been improved in broiler chicks (Najafzadeh et al. 2011). To the author knowledge, no work has been conducted on the effects of Echinacea in dogs in addition with vaccination. In this study, as part of a comprehensive study, the effects of long-term consumption of hydroalcoholic extract of *Echinacea purpura* in conjunction with vaccination on general condition, hematologic and biochemical parameters of dogs were evaluated.

Materials And Methods

In order to perform this study, 15 healthy adult dogs (1.5 to 2.5 years old) from unvaccinated mongrel breeds and both sexes were prepared. Dogs were kept in individual cages for two weeks in order to clinically examine for general health and prescribe anti-parasitic drugs. This study was approved by the ShahidChamran University of Ahvaz Care and Use Committee. Dogs were randomly divided into three equal groups of five, as follows. Dogs in group 1 received daily 100 mg/kg of *Echinacea purpurea* extract (Ahura Med, Iran) orally for 28 days. On the seventh day, a canine polyvalent vaccine containing modified live canine distemper virus, canine adenovirus type-2, and canine parvovirus (Hipra, Spain) were injected subcutaneously. Dogs in group 2 received the Echinacea purpura extract with the same dose mentioned in group 1 for the same period, and on the seventh day, one ml sterile saline subcutaneously. In dogs of group 3, on the seventh day, polyvalent vaccine was subcutaneously injected. In order to investigate the long-term effects of Echinacea on the health status of dogs, general condition of dogs including body weight, body temperature, heart rate and respiratory rate during study were measured. Whole blood was collected by cephalic venipuncture on days 0, 28 and 56 and evacuated into sterile clot tubes to isolate serum. Sera were processed routinely and stored at -20C. Complete counting of blood cells was done using automated hematology analyzer (Mindray, China) and differential leukocyte cell count was done on blood smears stained with Giemsa. Serum biochemical parameters including AST, ALT, BUN and creatinine, as well as total protein and serum albumin levels were measured using an automated biochemistry analyzer (Biotecnica, BT 1500, Italy) and commercial kits (Pars Azmun, Iran). SPSS software version 16 was used to analyze the data. Data were analyzed using analysis of variance with repeated measure and LSD test. The data were reported as mean ± standard error and the mean difference was considered as significant by $p<0.05$.

Table 1. Mean ± standard error of vital signs of dogs received *Echinacea purpurea*, polyvalent vaccine or their combination.

Parameters	Time	Group1 (Echinacea+Vaccine)	Group2 (Echinacea)	Group3 (Vaccine)
Heart rate min^{-1}	0	89.20±1.20	89.20±1.49	88.40±1.16
	28	88.00±1.67	88.00±1.09	88.00±1.10
	56	88.40±0.74	88.40±1.83	88.00±1.41
Respiratory $rate^{-1}$	0	20.40±0.51	20.20±0.86	20.20±1.07
	28	19.40±0.68	20.20±0.58	19.60±0.68
	56	21.00±0.71	20.00±1.10	20.80±0.58
Body Weight (kg)	0	20.46±1.29	19.92±1.32	20.24±0.73
	28	20.68±1.28	20.52±1.17	21.24±1.05
	56	20.16±0.67	21.06±1.57	20.20±0.52
Body temperature ºC	0	38.84 ±0.09	38.80±0.07	38.82±0.11
	28	38.84±0.08	38.90±0.09	38.88±0.07
	56	38.82±0.11	38.84±0.07	38.78±0.08

Table 2. Mean ± standard error of hematologic parameters of dogs received *Echinacea purpurea*, polyvalent vaccine or their combination.

Parameters	Time	Group1 (Echinacea+Vaccine)	Group2 (Echinacea)	Group3 (Vaccine)
RBC ($\times 10^6$ mm^3)	0 28 56	6.00±0.19 ^{Aa} 6.99±0.18 ^{Ab} 5.91±0.15 ^{Aa}	5.80±0.20 ^{Aa} 6.63±0.20 ^{Ab} ^{Bb}6.50±0.36	5.78±0.29 ^{Aa} 5.84±0.11 ^{Ba} 5.75±0.19 ^{Aa}
Hb (g/dl)	0 28 56	12.46±0.24 ^{Aa} 15.62±0.13 ^{Ab} 13.26±0.28 ^{Aa}	12.82±0.47 ^{Aa} 14.44±0.34 ^{Ab} 14.60±0.68 ^{Bb}	12.42±0.56 ^{Aa} 12.76±0.46 ^{Ba} 12.74±0.54 ^{Aa}
Hct (%)	0 28 56	43.24±1.58 48.24±0.81 42.00±1.18	41.86±1.47 46.10±1.22 45.92±1.95	42.52±2.70 42.44±1.84 40.56±1.69
WBC	0 28 56	10080.00 ±640.62 ^{a} 13440.00±358.60 ^{b} 11260.00±541.84 ^{a}	10100.00±492.95 ^{a} 13360.00±476.02 ^{b} 11320.00±269.07 ^{a}	9980.00±531.41 ^{a} 13000.00±461.51 ^{b} 11200.00±339.11 ^{a}
Lymphocyte	0 28 56	2160.00 ±229.34 ^{Aa} 3720.00±182.75 ^{Ab} 2300.00±234.52 ^{Aa}	1920.00±220.00 ^{Aa} 3460.00±203.96 ^{Ab} 2180.00±193.39 ^{Aa}	1880.00±583.09 ^{Aa} 1740.00±509.90 ^{Ba} 1800.00±316.22 ^{Aa}
Neutrophil	0 28 56	9768.80 ±1082.20 ^{Aa} 9295.20±787.67 ^{Aa} 9292.40±767.52 ^{Aa}	8759.20±1342.00 ^{Aa} 5104.00±572.34 ^{Bb} 9217.00±1082.40 ^{Aa}	9885.80±400.83 ^{Aa} 8921.40±837.05 ^{Aa} 9516.00±802.46 ^{Aa}

Different capital letter superscript indicate significant difference within groups ($p<0.05$). Different small letter superscript indicate significant difference within sampling days ($p<0.05$).

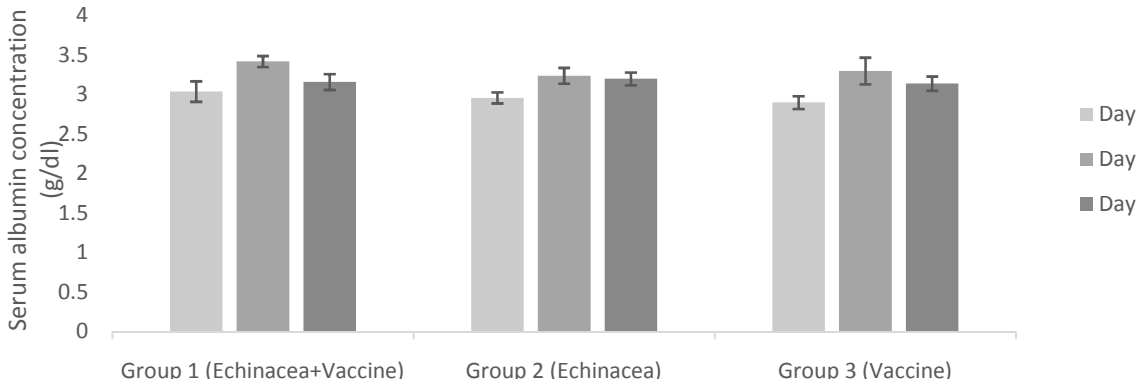

Fig 1.Mean ± SE of serum albumin concentration (g/dl) in different groups.

Table 3. Mean ± standard error of biochemical parameters of dogs received *echinacea purpurea*, polyvalent vaccine or their combination

Parameters	Time	Group1 (Echinacea+Vaccine)	Group2 (Echinacea)	Group3 (Vaccine)
AST (U/L)	0	19.20±3.53	21.60±2.11	16.60±1.33
	28	21.80±1.93	23.80±1.50	18.20±1.68
	56	22.00±1.51	22.60±3.94	28.00±5.50
ALT (U/L)	0	36.20 ±7.20	42.80±1.31	23.00±2.41
	28	36.20±3.25	36.20±3.38	38.20±8.75
	56	35.00±4.77	31.60±3.23	28.60±4.46
BUN (mg/dl)	0	39.60 ±2.86	32.20±2.67	35.80±3.43
	28	35.00±3.36	40.40±6.43	34.40±3.85
	56	38.40±1.50	41.20±3.81	38.40±1.21
Creatinine (mg/dl)	0	1.28 ±0.07	1.13 ±0.06	1.39 ±0.10
	28	1.10 ±0.07	1.09 ±0.08	1.11 ±0.05
	56	1.44 ±0.17	1.31 ±0.08	1.14 ±0.10

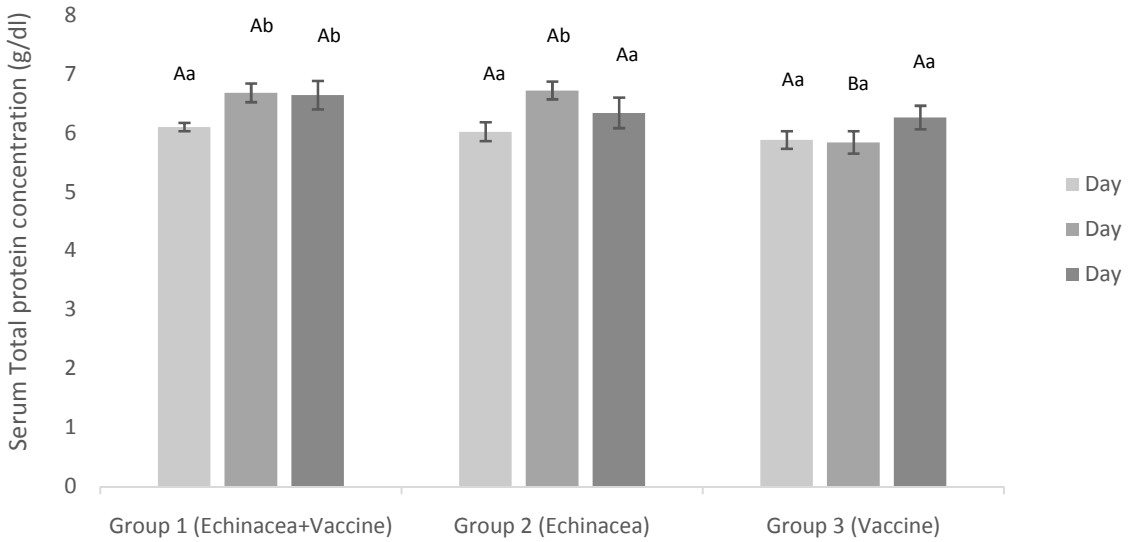

Fig 2: Mean ± SE of serum total protein concentration (g/dl) in different groups. Different capital letter superscript indicate significant difference within groups (p<0.05). Different small letter superscript indicate significant difference within sampling days (p<0.05).

Results

The results of long-term administration of Echinacea, polyvalent vaccine and their combination on body weight, body temperature, heart rate and respiratory rate are presented in Table 1. Statistical evaluation showed that there were no significant changes in heart rate, respiratory rate, body weight and body temperature on days 0, 28 and 56 (p> 0.05).Statistical analysis showed that the number of red blood cells and hemoglobin concentration in group 1 and group 2 had a significant difference with group 3. Accordingly, the number of red blood cells increased significantly in groups 1 and 2 on day 28 than group 3 (p=0.001). Also, on the day 28, the hemoglobin level was significantly increased in groups 1 and 2 as compared to group 3 (p=0.025). On the day 56, this increase was also seen in group 2 (p<0.05), but in group 1 it returned to the baseline

measurements. There are no significant differences between groups in the percentage of hematocrit at 0, 28, and 56 days ($p > 0.05$). An increase in the number of white blood cells in all three groups was observed in day 28, which was statistically significant ($p<0.001$). The number of lymphocytes increased significantly in day 28 in groups 1 and 2 ($p<0.001$). Also, the number of neutrophils in the day 28 of study in group 2 was significantly decreased ($p=0.03$). It should be noted that there was no difference in the number of monocytes, eosinophils and basophils among the groups ($p > 0.05$). The hematological data of all three groups on days 0, 28 and 56 of the study are shown in Table 2.The results of biochemical evaluation (Table 3) showed no significant differences in serum concentrations of aspartate aminotransferase, alanine aminotransferase, blood urea nitrogen and creatinine ($p>0.05$).Based on statistical analyses, there was no significant difference in the level of albumin between groups in days 0, 28 and 56 ($p > 0.05$). There was no significant difference in serum total protein level between groups in day 0 ($p>0.05$). On day 28, total protein concentration increased significantly in groups 1 and 2 ($p=0.03$). This increase was still evident in group 1 until day 56 ($p<0.05$). Changes in serum albumin and total protein concentrations are shown in figures 1 and 2.

Discussion

The effect of *Echinacea purpurea* has been considered on the immune system responses of human and animals in several studies. This study which is a part of project that evaluates the immunomodulatory effect of Echinacea on vaccination, the systemic effects of long-term feeding of this herb on general condition, hematologic and biochemical parameters were investigated. In the present study, there were no significant changes in body weight, body temperature, heart rate and respiratory rate during the study. Torkan et al. (2015) investigated the effect of oral administration one ml of Hydroetanolic extract of Echinacea 5% twice a day and for two months on the immune system of 14 mixed breed male dogs. They did not report any changes in dog's vital signs, which is consistent with the present study. Based on the results of this study, Echinacea alone and in combination with polyvalent vaccine could increase the number of red blood cells and hemoglobin concentration in dogs significantly. In a similar study, the effect of oral administration of the extract of *echinaceaangustifolia* root (standardized on the basis of 4% acinar oxide) on equine hematologic parameters was investigated (O`Neill et al. 2002). In that study, 30 ml of the extract of herb twice daily was added to the diet of each horse for 42 days. They found that echinacea increased the level of hemoglobin and the number of red blood cells, which is consistent with this study. Sharma et al. (2006) stated that Echinacea extracts increased the nuclear content of more than 30 transcription factors, including the 12 pro-inflammatory factors. Also, hematinic properties of echinacea have been reported in rats (Chow et al. 2006) and rainbow trout (Oskoii et al. 2012). On the other hand, heterogeneity findings in pigs have also been reported. In other words, echinacea has not been able to significantly alter hematologic parameters in pigs. However, the researchers believe that echinacea should be added to diet of pigs as a nutritional supplement to stimulate the immune system and increase the conversion rate of food to meat (Maass et al. 2005). A significant increase in the number of lymphocytes due to echinacea compared with the polyvalent vaccine in this study is consistent with the findings of other researchers. See et al. (1997) showed that the *Echinacea purpurea* extract could be considered as an immunostimulant in vitro. So that the echinacea in addition to increase the number of lymphocytes, improved their function in healthy people as well as with acquired immune deficiency. SteinMüller et al. (1993) showed that polysaccharides isolated from cell cultures of *Echinacea purpurea* activate human and murine phagocytes, induced an earlier influx of neutrophil granulocytes in immunosuppressed mice and increased their resistance against lethal infections with the *Listeria monocytogenes* and *Candida albicans*.Significant decrease in the neutrophil counts in echinacea receiving dogs in this study could be due to increased neutrophilic adhesion to blood vessels and their migration into tissue (O`Neill et al. 2002). Roesler et al. in 1991 reported that *Echinacea purpurea* extract could be activated macrophages from different organ origin to produce IL-1, TNF alpha and IL-6 and to produce elevated amounts of reactive oxygen intermediate in vitro. Furthermore, in vivo the substances could induce increased proliferation of phagocytes in spleen and bone marrow and migration of granulocytes to the peripheral blood. O'Neil et al. (2002) demonstrated that treatment with Echinacea increased phagocytic ability of isolated neutrophils, boosted peripheral lymphocyte counts and appeared to stimulate neutrophil migration from peripheral circulation into the tissues of horses. This suggests that the stimulated neutrophils were better suited to consuming foreign particles, thereby improving the animal`s defense against opportunistic pathogens. Significant increase in the white blood cells count in all dogs in the present study could be due to the hematopoietic properties of echinacea (O`Neill et al. 2002, Torkan et al. 2015). In the polyvalent vaccine recipient group, this increase can be related to the immunosuppression after vaccination. A decline in T-cell-mediated immunity and transient state of immunosuppression after immunization with a modified live vaccine has been reported in dogs, followed by an increase in the number of white blood cells (Strasser et al. 2003).No significant changes in the number of monocytes, eosinophils and basophils of dogs in present study were consistent with other

works in horses, fishes, and dogs (O`Neill et al. 2002, Torkan et al. 2015, Oskoii et al. 2012).The use of echinacea in this study did not result in any side effects in dogs. In this regard, no abnormalities were observed in serum aspartate aminotransferase, alanine aminotransferase, albumin, blood urea nitrogen and creatinine. Single oral or intravenous doses amounting to many times the human therapeutic dose of the expressed juice of *Echinacea purpurea* proved virtually non-toxic to rats and mice. After 4 weeks laboratory tests and necropsy findings gave no evidence of any toxic effects in rats (Mengs et al. 1991). Total plasma protein consists of albumin and globulin (α, β, and γ). Therefore, a significant increase in total protein caused by administration of echinacea in dogs and the lack of changes in serum albumin levels during the study can be indicative of an increase in blood globulin levels. Since an important part of the globulins is gamma globulin (Morag 2002), the increase in total plasma protein can be due to gamma-globulin increase, which a closer examination of these components by electrophoresis can be considered in future studies. As a final conclusion in the present study, it can be concluded that *Echinacea purpurea* extract as a hematinic agent in dogs has been associated with an increase in the number of red blood cells and hemoglobin levels. Its long-term use has also led to an increase in the total plasma protein, lymphocyte and neutrophil counts, without significant changes in the liver and kidney markers.

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