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Evaluating the effect of oral administration of *Echinacea* hydroethanolic extract on the immune system in dog

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Summary

1. This study was designed to evaluate the effects of oral administration of *Echinacea* hydroethanolic extract on the dog's immune system.
2. The study was performed on 14 dogs that were referred to the veterinary clinic. These dogs were randomly allocated to two equal treatment groups. The first group received 1 ml of 5% *Echinacea* hydroethanolic extract two times a day for 2 months, and the second group received a placebo (water). To do haematology and immunology tests, the dogs were bled on days 0, 30 and 60. Blood tests, including packed cell volume (PCV), haemoglobin (Hb), red blood cell count (RBC), white blood cell count (WBC), counting neutrophils (Nut), lymphocytes (Lym), monocytes (Mon), eosinophils (Eos), basophils (Baso) and B cell, were performed. Furthermore, safety factor IgM and per cent of phagocytosis and phagocyte were measured from the blood sample.
3. The results showed that in the group which received *Echinacea* PCV, Hb, RBC count, WBC count, Lym, Nut, the per cent of phagocytosis and IgM significantly increased ($P < 0.05$). Moreover, positive effects of *Echinacea* plant on the immune system were observed. There was a significant change in HTC, RBC, Hb over time in the group that received *Echinacea* and the per cent of phagocytosis and IgM ($P < 0.05$).
4. The study establishes that these extracts might have appreciable immunostimulatory activity. However, further studies are required to confirm these findings.

Keywords: *Echinacea*, oral administration, dog, immune system

Introduction

The use of liquid extracts of *Echinacea* has increased recently and most often used as immunostimulating agents for the treatment (Hermann *et al.*, 2003) and prevention of various infectious disorders in human medicine (Wolfram & Hans-Helge, 1999). This is due to an increase in the clinical importance of herbal drugs in modern medicine with considerable attention being paid to the use of plants as a source of immunomodulators being at the centre stage. Several medicinal herbs have shown to promote immunity in different ways; they have shown to augment specific cellular and humoral immune response (Duke, 1985).

Immunomodulators are agents that can modulate the immune response, and their effect may be stimulatory or suppressive (Ghonime *et al.*, 2011). *Echinacea* extracts exhibit that potential. Rehman *et al.* (1999) (Rehman *et al.*, 1999) showed an increase in primary and secondary IgG response in rats treated with *Echinacea*. A few scientific studies have assessed the efficacy of Echinacea *in vivo* with varying results (Grimm & Mu¨ ller, 1999; Turner *et al.*, 2000). Therefore, there is need to assess the effects of *Echinacea* hydroethanolic extract on dogs as an animal model in assessing its efficacy as an immunostimulant. This study was designed to evaluate the effects of oral administration of *Echinacea* hydroethanolic extract on the dog's immune system.

Materials and methods

Animals

All experiments were carried out under the ethical guidelines of the Islamic Azad University of Shahrekord Branch, for the care and use of animals (Ernest *et al.*, 1993).

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The study was performed on 14 male dogs of mixed breeds that were randomly allocated to two equal treatment groups. All dogs were subjected to clinical examination and housed under uniform environment after being treated for internal parasites. The first group received 1 ml of 5% *Echinacea* hydroethanolic extract two times a day for 2 months, and the second group received a placebo (water) instead of *Echinacea* extract.

Preparation method for Echinacea extract

Fifty grams of powdered plant was added to 700 ml of 50% ethanol (350 ml distilled water and 350 ml ethanol), and Soxhlet apparatus was used to prepare hydroethanol extract. The solvent was filtered under reduced pressure. The plant ingredient concentration in the final extract was adjusted to the required concentration by adding distilled water to the dried extract. The extract was prepared each week and stored in a refrigerator.

Sample collection and analyses

The dogs were bled on days 0, 30 and 60 for haematology and immunology tests, and every blood sample was divided into two equal volumes for further analyses. The blood tests that were carried out include packed cell volume (PCV), haemoglobin (Hb), red blood cell count (RBC), white blood cell count (WBC), counting neutrophils (Nut), lymphocytes (Lym), monocytes (Mon), eosinophils (Eos) and basophils (Baso).

Statistical analysis

Statistical analysis focused on mean analysis for repeated measures that is before intervention, 30 days and 60 days. The following model was used:

$$Y_{ijkl} = \mu + \tau_i + B_j + T_k + (\tau BT)_{ijkl} + \varepsilon_{ijkl}$$

where Y_{ij} is the response measured on the ith treatment, i = [PCV, Hb, RBC count, WBC count, counting Nut, Lym, Mon, Eos, Baso, B and Cell], on the jth dog, at the kth time, μ is the overall mean, τ is the mean effect of treatment, B is the random subject effect, T_k is the time effect, τBT is the interaction effect and ε is the error.

Results

The total sample size was 14, and each treatment group comprised of seven dogs. Body temperature and respiratory rate of the two groups were significantly different before the intervention Table 1 ($P < 0.05$). Heart rate was higher in the group that was selected to receive *Echinacea* but not significantly different from the group that received the placebo ($P > 0.05$). The results showed that in the group which received *Echinacea* PCV, Hb, RBC count, WBC count, Lym, Nut, the per cent of phagocytosis and IgM significantly increased (Table 2 and 3) ($P < 0.05$). Also, the results indicated effects of *Echinacea* plant on the immune system. There was a significant change over time in the group that received *Echinacea* on HTC, RBC, Hb and the per cent of phagocytosis and IgM ($P < 0.05$).

Table 1 The mean ± SD of vital signs of two groups before intervention

Variable	Group	Number of dogs	Mean
Heart rate min^{-1}	*Echinacea*	7	87.9 ± 12
	Placebo	7	84.7 ± 8.7
Body temperature °C	*Echinacea*	7	37.76^{b} ± 0.6
	Placebo	7	38.47^{a} ± 0.4
Respiratory rate min^{-1}	*Echinacea*	7	20.1^{b} ± 2.5
	Placebo	7	25.3^{a} ± 2.6

Different superscript letters within the same column indicate significant difference ($P < 0.05$).

Discussion

Burger *et al.* (1997) (Burger *et al.*, 1997) and See *et al.* (1997) (See *et al.*, 1997) observed that extracts from *Echinacea* have non-specific immunostimulatory properties *in vitro* including increased phagocytosis, cytokine production and natural killer cell activity. The plant and its extracts have been shown to stimulate phagocytosis *in vitro* and *in vivo* in murine (Melchart *et al.*, 1995). Roesler *et al.* (1991) (Roesler *et al.*, 1991) confirmed activation of human phagocytic function both *in vitro* and *in vivo*.

Information generated in past research suggests that the immunostimulatory activity of *Echinacea* depends on the combined action of caffeic acid derivatives and alkylamides (Bauer 1998; Hermann *et al.*, 2003). Moreover, many pharmacological compounds have been isolated from *Echinacea* (San Feliciano *et al.*, 1993), and several constituents are alleged to be immunologically active, including polysaccharides and glycoproteins (Bauer *et al.*, 1988). Sloley *et al.* (2001) (Sloley *et al.*, 2001) showed that phenylpropanoid glycosides, which are constituents of certain *Echinacea* species, possess antiviral properties and are antioxidants and free radical scavengers and inhibit Fe^{2+}-induced lipid peroxidation.

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Table 2 Comparison of mean ± SD of blood parameters before and after intervention

Variable	Treatment	Before	1 month	2 month
HTC	*Echinacea*	$43.43^{b} \pm 1.11$*	$47.71^{a} \pm 0.606$**	48.14 ± 0.857**
	Placebo	$47^{a} \pm 3.97$	$46.29^{b} \pm 4.539$	50 ± 1.397
Red blood cell count (RBC)	*Echinacea*	$7.36^{b} \pm 0.143$*	$7.86^{a} \pm 0.053$**	$7.94^{a} \pm 0.084$**
	Placebo	$7.47^{a} \pm 0.465$	$7.50^{b} \pm 0.493$	$7.46^{a} \pm 0.178$
Haemoglobin (Hb)	*Echinacea*	$14.70^{a} \pm 0.235$	$15.34^{a} \pm 0.043$	$15.10^{a} \pm 0.136$
	Placebo	$14.26^{b} \pm 0.612$	$14.56^{b} \pm 0.581$	$14.42^{b} \pm 0.274$
White blood cell count (WBC)	*Echinacea*	$12990^{a} \pm 445.67$*	13290 ± 441.65**	13550 ± 408.54**
	Placebo	$11580^{b} \pm 233.467$	11890 ± 535.079	11950 ± 328.494
Neutrophils (Nut)	*Echinacea*	58.143 ± 3.181*	62.286 ± 1.96**	56.714 ± 2.579*
	Placebo	63.714 ± 3.746*	61.143 ± 3.508*	57.429 ± 3.872**
Band	*Echinacea*	2.286 ± 0.747	1.571 ± 0.297	1.571 ± 0.429
	Placebo	1.714 ± 0.36	1.714 ± 0.184	1.143 ± 0.508
Lymphocytes (Lym)	*Echinacea*	27.143 ± 2.539*	31.714 ± 2.942**	34.286 ± 4.144**
	Placebo	32.571 ± 2.626*	31.714 ± 2.109*	27.143 ± 3.068**
Monocytes (Mon)	*Echinacea*	2.714 ± 0.565	2.857 ± 0.34	3.857 ± 0.34
	Placebo	3.286 ± 0.522	3.429 ± 0.369	2.857 ± 0.261
Eosinophils (Eos)	*Echinacea*	4.143 ± 0.738	3.857 ± 1.122	4.371 ± 0.528
	Placebo	3.714 ± 1.04	3 ± 0.65	4 ± 0.535
Basophils (Baso)	*Echinacea*	$0.313^{b} \pm 0.143$	0.557 ± 0.143	0.571 ± 0.297
	Placebo	$0.429^{a} \pm 0.202$	0.286 ± 0.184	0.571 ± 0.297

Different superscript letters within the same column indicate significant difference ($P < 0.05$).
*; **; *** = significantly different means in the same row.

Table 3 Comparison of phagocytes, phagocytosis and IgM before and after intervention

Variable	Treatment	Before	30 days	60 days
Phagocytes	*Echinacea*	25.857 ± 2.604*	27.286 ± 2.884**	28.462 ± 2.07**
	Placebo	34.286 ± 2.427*	32.571 ± 2.776*	27.286 ± 1.886**
Phagocytosis	*Echinacea*	25.429 ± 3.747	25.143 ± 2.385	23.857 ± 2.064
	Placebo	30.714 ± 4.714	23.143 ± 2.721	26.429 ± 1.478
IgM	*Echinacea*	$138.714^{b} \pm 3.242$*	159.857 ± 13.674**	182.857 ± 13.956***
	Placebo	$150.286^{a} \pm 18.774$	181.714 ± 7.63	186.429 ± 6.679

Different superscript letters within the same column indicate significant difference ($P < 0.05$).
*; **; *** = significantly different means in the same row.

The haemoglobin levels were significantly increased in the trial by *Echinacea* extract. This is supported by Anon (1989) (Anon, 1989) who concluded that *Echinacea* extract behave as an agent that improves the quality of blood by increasing haemoglobin levels and the number of erythrocytes therefore, considered to improve parameters of exercise physiology and performance.

Increase in the number of lymphocytes during the treatment phase of the study support the information generated in other investigations (See *et al.*, 1997; Steinmuller *et al.*, 1993) which suggests that *Echinacea* behaves as an immune system stimulant. In addition, previous studies have demonstrated that *Echinacea* has an enhancing effect on lymphocyte function and proliferation (See *et al.*, 1997). Furthermore, Echinacea extracts showed protection of immunosuppressed mice against systemic infections with stimulation of macrophage and neutrophil function (Steinmuller *et al.*, 1993).

Increase of neutrophil counts was achieved only after the first month and then decreased after the second month on *Echinacea* treatment group, thereby raising the question as to what other external factors may have contributed to the change. However, Melchart *et al.* (1995) (Melchart *et al.*, 1994, 1995) and O'neill *et al.* (2002) demonstrated the effect of *Echinacea* on the capacity of neutrophils to ingest more foreign particles and stimulatory effect on these cells by improving phagocytic function.

The changes that occurred to the blood parameters measured in the current study are strongly due to the effects of *Echinacea* which acted as an immunomodulator by activating cytotoxic effecter cells such as cytotoxic T lymphocytes, natural killer (NK) cells, lymphocytes, macrophages and

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activated neutrophils as observed by Ghonime *et al.* (2011) (Ghonime *et al.*, 2011).

Administration of *Echinacea* showed increased number of the total WBC count. Similar increase in WBC count was obtained by plant extracts of *Silene nocturna, Nigella sativa* and *Matricaria chamomilla* (Ghonime *et al.*, 2011) *and Withania somnifera* (Davis & Kuttan, 2000). This indicates they can stimulate the hemopoietic system. Although significant patterns were observed, nevertheless, one limitation of the study was the insufficient sample to detect small to moderate differences in the parameters measured between the *Echinacea* and the placebo groups. However, the sample size was small and that might have resulted in large discrepancies observed between the two treatments.

Conclusion

The study establishes that these extracts have appreciable immunostimulatory activity. However, further studies are required to confirm these conclusions.

Conflict of interest

The authors declared no conflict of interests with respect to the research, authorship and/or publication of this article.

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