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RESEARCH ARTICLE

Immunostimulatory Effects of *Aloe vera* and *β-Glucan* on Cellular and Humoral Immune Responses Following Vaccination with Polyvalent Vaccines in Dogs

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Summary

The aim of this study was to evaluate the effects of *Aloe vera* and *β-Glucan* on lymphocyte subsets, haematological parameters and immunglobulin concentrations following vaccination in dogs. For this purpose, totally 20 street dogs were used. The animals were allocated into five groups. Group 1 consisted of only polyvalent vaccine applied dogs. Group 2 and 3 were only *Aloe vera* and *β-Glucan* applied dogs, respectively. Group 4 and 5 were consisted of polyvalent vaccine in addition to *Aloe vera* and *β-Glucan* applied dogs, respectively. Blood samples were collected before vaccination and after vaccination (AV). Although platelet (PLT) counts in group 1 decreased AV, in all other groups (group 2, 3, 4, 5) it increased. White blood cell (WBC) and peripheral blood mononuclear lymphocyte (PBML) counts (in all groups), peripheral blood polymorphonuclear lymphocyte (PBPL) counts (group 1, 2, 4, 5), neutrophil (group 1, 4, 5), monocyte (group 2, 3, 5), packed cell volume (PCV) ratios (group 2 and 3) and haemoglobin (HGB) concentrations (group 3) increased AV. But, lymphocyte ratios in all groups did not change AV. CD8 T lymphocyte ratios in all groups increased at 7th and 14th days AV. Although CD3 T lymphocyte ratios in group 1 increased at 7th day AV, CD4 T and B lymphocytes ratios did not change AV period. But, both CD3 and CD4 T lymphocytes ratios in all other groups increased at 7th and 14th days AV. Moreover, B lymphocytes ratios in all groups except for group 1 increased AV. On the other hand, B lymphocytes ratios in group 2 and 3 increased only 7th day AV, while this increase in group 4 and 5 were present on both 7th and 14th days AV. Serum IgM concentrations in group 1 increased only 7th day AV, whereas its levels did not change in group 2 and 3. Serum IgG concentrations in group 1, 2 and 3 increased only 14th day AV. But, serum IgM and IgG concentrations in group 4 and 5 increased at 7th and 14th days AV. Administration of *Aloe vera* and *β-Glucan* after vaccination in dogs may stimulate both cellular and humoral immun responses. Additionally, it might have restorative effect on PLT counts.

Keywords: *Aloe vera, β-Glucan, Dog, Vaccination, Immunity*

Köpeklerde Karma Aşı ile Aşılama Sonrası Hücresel ve Humoral İmmun Cevaplar Üzerinde *Aloe Vera* ve *β-Glucan*'ın İmmunostimülatör Etkileri

Özet

Bu çalışmada köpeklerde aşılama sonrası lenfosit alt tipleri, hematolojik parametreler ve immunglobulin konsantrasyonları üzerinde *Aloe vera* ve *β-Glucan*'ın etkilerinin değerlendirilmesi amaçlandı. Yirmi sokak köpeği kullanıldı. Hayvanlar beş gruba ayrıldı. Grup 1'deki köpeklere sadece karma aşı uygulandı. Grup 2 ve 3'teki köpeklere sırasıyla sadece *Aloe vera* ve *β-Glucan* uygulandı. Grup 4 ve 5'teki köpeklere ise karma aşıya ilaveten *Aloe vera* ve *β-Glucan* uygulandı. Aşılama öncesi ve aşılama sonrası (AS) kan örnekleri alındı. Platelet (PLT) sayıları grup 1'de AS azalmasına rağmen, diğer tüm gruplarda (grup 2, 3, 4, 5) arttı. Total lökosit (WBC) ve periferal kan agranülosit (PBML) sayıları (tüm gruplarda), periferal kan granülosit (PBPL) sayıları (grup 1, 2, 4, 5), nötrofil (grup 1, 4, 5), monosit (grup 2, 3, 5) ve hematokrit (PCV) oranları (grup 2, 3) ile hemoglobin (HGB) konsantrasyonları (grup 3) AS'da arttı. Fakat, lenfosit oranları AS'da tüm gruplarda değişmedi. CD8 T lenfosit oranları tüm gruplarda AS 7 ve 14. günlerde arttı. CD3 T lenfosit oranları grup 1'de AS 7. günde artmasına rağmen, CD4 T lenfosit ve B lenfosit oranları AS periyotta değişmedi. Fakat, CD3 ve CD4 T lenfosit oranları diğer tüm gruplarda AS 7. ve 14. günlerde arttı. Üstelik, B lenfosit oranları AS'da grup 1 hariç diğer tüm gruplarda arttı. Ayrıca, B lenfosit oranları grup 2 ve 3'de sadece AS 7. günde artarken, grup 4 ve 5'de AS hem 7. hemde 14. günde arttı. Serum IgM konsantrasyonları grup 1'de sadece AS 7. günde artarken, grup 2 ve 3'de değişmedi. Serum IgG konsantrasyonları grup 1, 2 ve 3'de sadece AS 14. günde arttı. Fakat, serum IgM ve IgG konsantrasyonları grup 4 ve 5'de AS 7. ve 14. günde arttı. Köpeklerde aşılama sonrası *Aloe vera* ve *β-Glucan* uygulamasının hücresel ve hümoral immun cevapların her ikisini de stimüle edebileceği ve PLT sayıları üzerinde restoratif etkili olabileceği kanısına varıldı.

Anahtar sözcükler: *Aloe vera, β-Glucan, Köpek, Aşılama, İmmünite*

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INTRODUCTION

Vaccination against several pathogens using various type of vaccines have commonly been used in veterinary and human medicine [1-8]. Despite a transient state of immunosuppression after vaccination as reported in a few studies [7,9-12], dogs are routinely vaccinated against various diseases including canine distemper virus (CDV), canine adenovirus type-1 (CAV-1), canine adenovirus type-2 (CAV-2), canine parainfluenza (CPI) and canine parvovirus (CPV) [1-7], *Bordetella bronchiseptica, Borrelia burgdorferi, Leptospira spp., Coronavirus,* and *Giardia spp.* with standard vaccines and severe diseases do not generally occur [1-4,6-8]. In most of these studies virus induced immunosuppression have been investigated with regard to mono-valent vaccines such as CDV, CPV, CAV-1 and CAV-2 [7,9-12]. Immunosuppressive role of polyvalent vaccines have also been studied in recent years [4,6,13]. It is also reported that maximum vaccination schedules do not appear to be more effective or more immunosuppressive than minimum vaccination schedules [13]. Furthermore, it is declared that the observed changes after polyvalent vaccination might be reflected a shift from cell-mediated to humoral immunity after immunization and probably are immunomodulative rather than immunosuppressive [4,6]. Eventhough, vaccination of dogs lead to alterations [4,6,13] or adverse effect [1,2,9,14] in immune parameters in the post-vaccinal period. Vaccination and procedural manipulations (e.g. injections) itself represents a stress on the immune system of dogs [6,14] as physical and psychologic stresses such as handling, crowding, fear etc. may affect an animal's immune response. Stress-mediated effects on immune function are most likely hormonally mediated. Especially, corticosteroids are most likely involved in mediating stress responses [9,14-16] as it is reported that plasma cortisol response to handling associated is initiated with at 3 min of the commencement of handling [17].

Recently, there has been an increasing interest of using complementary and alternative medicine. Immuno-modulatory polysaccharide represents one of the many biological response modifiers (BRMs) which can be prepared from bacteria, fungi, and plants. The poly-saccharides are thus very diverse in their components and linkages except those derived from fungi and aloe. In fungi, the majority polysaccharide BRMs are β-(1→3)-D-Glucans and those from yeast are α-D-Mannan and Glucomannan [18]. Natural β-(1,3)- D-Glucans can be isolated from almost every species of yeast. Especially, β-(1,3)-D-Glucan derived from *Saccharomyces cerevisiae* has been the most extensively studied [19]. Polysaccharide BRMs derived from *Aloe vera* are mainly mannose polymer with β-(1→4)-D-linkage [18]. Of the more than 360 Aloe species known, *Aloe barbedensis* Miller is the most widely used both commercially and for its therapeutic properties [20]. Although numerous routes for administration of *Aloe vera* and β-*Glucan* products existed, in the last decade oral application represents the most convenient route [19,21-23]. In addition, promoters offer a number of formulations that are widely available for consumption at various concentrations in liquid, powder, and tablet form [19-21,24-26].

Immunostimulants are widely used in animals for health management [19,20,25-27]. Some plant polysaccharides (such as β-*Glucan* and *Aloe vera*) are well known to possess immunoregulatory and/or immunostimulatory effects [19,20,27-29]. β-*Glucan*'s and *Aloe vera*'s role as a biologically active immunomodulator have been well documented for years [28,30,31]. Especially, these products have been extensively studied for their immunological effects [19,28,30]. In these studies, it was demonstrated that either particulate or soluble forms, exhibited strong immunostimulating and/or immunomodulatory properties in all tested animal species [18,19,24,27,28,31].

Administration of *Aloe vera* and β-*Glucan* have been universally demonstrated to result in marked increase in phagocytic and proliferative activity of the reticulo-endothelial system [21,26,28,32-35]. These products can modulate and stimulate both humoral and cellular immunity and it also stimulates proliferation of murine pluripotent hematopoietic stem cells, granulocyte macrophage colony-forming cells, and cells forming myeloid and erythroid colonies [21,24,25,28,31,33,36]. It is demonstrated that immunomodulating and/or immunostimulating effects of these products are dependent on the activation of the innate immune cells (macrophages, neutrophils, lymphocytes and NK cells), synthesis and release of cytokines (TNF-α, IFN-α, IFN-γ, IL-1, IL-2, IL-6, IL-8), generation of enhanced cell-mediated responses, and induction of nitric oxide production [18-20,24,25,28,30-32,37].

Besides the role in cellular immunity, researchers also found that *Aloe vera* and β-*Glucan* induced antibody responses. The effects on antibody responses were proved by the significant increase in the number of B lymphocytes forming both IgM and IgG antibodies [21,25,30,31]. Moreover, it is reported that this products can be used as an effective adjuvant when administered with antigen which is an immune enhancer that augments immune response [21,25,30,33,37,38]. Possible use of this products in preventing hemopoietic depletion or enhancing hemopoietic recovery have also been reported [23,29,33]. Furthermore, the immunomodulators were found to exert a restorative effect on the values of reduced cell populations (T and B) such as suppression and/or deficiencies of the immune system in the host organism [18,24,30,38,39].

Although there is a general consensus on the multiple immunomodulatory and/or immunorestorative activities of *Aloe vera* and β-*Glucan*, the effects of these substances on immune parameters following immunization with polyvalent vaccines in dogs have not yet been reported. Therefore, in the present study, the immunostimulatory effects of orally administered *Aloe vera* and β-*Glucan* on a panel of immune parameters, including peripheral blood lymphocyte subsets, haematological parameters and serum immunglobulin concentrations will be investigated in polyvalent vaccine applied dogs.

MATERIAL and METHODS

Animals and Study Design

A total of 20 street dogs (10 female and 10 male) aged between 6 and 12 months were used. The vaccination history of the dams was unknown. For physiological adaptation, the animals were kept in the same environment at least one month before the study. During this period, antiparasitic theraphy was also applied. The dogs were undergone a routine medical examination prior to admission into the study and blood samples were analyzed in a routine haematology and biochemistry panel.

The animals were allocated into five groups 4 animals in each group (2 male and 2 female). Each group was housed in a separate pen. Group 1 consisted of only polyvalent vaccine applied dogs. Group 2 and 3 were only *Aloe vera* and β-*Glucan* applied dogs, respectively. Group 4 and 5 were consisted of polyvalent vaccine in addition to *Aloe vera* and β-*Glucan* applied dogs, respectively. The polyvalent vaccine (Vanguard® Plus 5/ L- Pfizer/Turkey) was a mixture of modified live virus (CDV, CPV, CAV-1, CAV-2 and CPI) and bacterin of *Leptospira canicol*a and *icterohaemorrhagiae,* and was injected subcutaneously.

Aloe vera juice obtained from *Aloe barbadensis* Miller (ALOVA-Australian Import Traders Gmbh) were given to group 2 and 4 orally at the dose of 5 ml/kg/day (for 14 days). This juice was provided by Doctor Klaus (IASC/Köln/Germany). *β-Glucan* (1,3-1,6- β-D-Glucan), in the microparticulate form, prepared from *S. cerevisiae* yeast (Imuneks® 10 mg, Mustafa Nevzat Drug Company, Turkey) was given to Group 3 and 5 orally at the dose of 3 mg/kg/day (for 14 days).

Collection of Samples

Blood samples were collected from v. sephalica antebrachii into the tubes with and without anticoagulant before vaccination (BV) and after vaccination (AV) (7th and 14th days). For biochemical analysis, the serum was separated by centrifugation at 3000 rpm/minute for 10 min and stored at -20°C until analysis.

Haematology

Haematological parameters included haemoglobin (HGB), packet cell volume (PCV), white blood cell (WBC), platelet (PLT), peripheral blood polymorphonuclear leukocytes (PBPL) and peripheral blood mononuclear leukocytes (PBML) counts: These parameters were determined using QBCvetautoreader® cell counter (IDEXX). A differential leukocyte counts was performed on Giemsa-stained blood smears (100 cells counted per slide).

Flow Cytometric Analysis

Monoclonal antibodies and negative controls used to quantitate the canine lymphocyte subsets were purchased from Serotec (Oxford, UK). The antibodies were specific for the following lymphocyte subsets: Anti-CD3 (CA17.2A12, T lymphocytes), anti-CD4 (YKIX 302.9, helper T cells), anti CD8 (YCATE 55.9, cytotoxic T cells), and anti-CD21 (CA2.1D6, B lymphocytes). The lymphocyte subsets were enumerated using the EPICS- XL® flow cytometer (Coulter). Appropriate negative controls were included to correct background fluorescence. Data were expressed as the percentage of positive-staining cells corrected for cells stained non-specifically with the secondary antibody.

Immunglobulin Determinations

Serum immunglobulin (IgG, IgM and IgA) concentrations were measured by ELISA technique (Microplate Reader® - DAS) using commercial test kit for IgA, IgG and IgM (MCA630®, Serotec), as previously described [40].

Statistical Analysis

Statistical differences between means of parameters within all five groups were evaluated by repeated measures of one-way variance analysis (ANOVA). Values with significant differences determined on ANOVA were subjected to student's t test to determine differences between the days in all groups. Analysis was performed using the SPSS 10.0 software. Statistical significance was set at $P<0.05$. All data were expressed as means±SEM.

RESULTS

Haematology

WBC and PBPL counts (7th and 14th day), PBML counts and neutrophil (NEUT) ratios (7th day) in group 1 increased AV compared to BV. But, only WBC counts in group 1 decreased on day 14 AV compared to the values on day 7 AV. PLT counts in group 1 decreased on day 7 and 14

AV compared to the values obtained BV *(Table 1)*.

WBC, PBPL and PBML counts (7th and 14th day), monocyte (MON) ratios (7th day), PCV ratios and PLT counts (14th day) in group 2 increased AV compared to BV. But, eosinophil (EOS) ratios in group 2 decreased only on day 7 AV compared to BV *(Table 1)*.

WBC, PBML, PLT counts, MON and PCV ratios, HGB concentrations in group 3 increased only on day 14 AV compared to BV, whereas EOS ratios decreased both 7th and 14th days AV *(Table 1)*.

WBC, PBPL, PBML, PLT counts (7th and 14th day) and NEUT ratios (only 7th day) in group 4 increased AV compared to BV *(Table 1)*.

WBC and PBPL counts (7th and 14th day), PBML, PLT counts and MON ratios (only 7th day), NEUT ratios (only 14th day) in group 5 increased AV compared to BV. But, PBML, PLT counts and MON ratios in group 5 was not statistically significant on day 14 AV compared to BV *(Table 1)*. Lymphocyte (LYM) ratios in all groups did not change AV period *(Table 1)*.

Lymphocyte Subsets

Although CD3 T lymphocyte ratios in group 1 increased only on day 7 AV compared to BV, its ratios in all other groups increased in 7th and 14th day AV. But, its ratios only in group 1 was determined to decrease at 14th day AV compared to 7th day AV. CD4 T lymphocytes ratios in all other groups except for group 1 increased both on days 7 and 14 AV compared to BV. However, CD8 T lymphocytes ratios in all groups increased both on days 7 and 14 AV compared to BV. B lymphocytes ratios in group 2 and 3 increased only on day 14 AV compared to BV. But, its levels in group 4 and 5 increased both on day 7 and 14 AV. However, B lymphocyte ratios in group 1 did not change AV compared to BV *(Table 2)*.

Serum Immunglobulin Concentrations

Serum IgG concentrations in group 1, 2 and 3 increased only on day 14 AV compared to BV, whereas its levels in group 4 and 5 increased both on day 7 and 14 AV. Serum IgM concentrations in group 1 increased only on day 7 AV compared to BV, whereas its levels in group 4 and 5 increased both on day 7 and 14 AV. But, serum IgM

Table 1. *Effects on haematological parameters of Aloe vera and β-Glucan administration alone or after polyvalent vaccine application in dogs (Mean±SEM)*

Tablo 1. *Köpeklerde tek başına ve karma aşı uygulaması sonrası Aloe vera ve β-Glucan uygulamalarının hematolojik parametreler üzerindeki etkileri (Ortalama±Standart Hata)*

Parameters	DAYS	GROUP 1	GROUP2	GROUP 3	GROUP 4	GROUP 5
WBC (x10⁹/L	Day 0	10.50±0.39	10.87±0.41	9.65±0.71	10.32±0.53	10.80±0.25
	Day 7	14.70±0.29^{c}	14.55±0.37^{c}	10.75±0.32	15.40±0.39^{c}	13.17±0.36^{b}
	Day 14	12.80±0.60^{by}	13.20±0.44^{b}	11.10±0.66^{a}	16.30±0.44^{c}	12.40±0.27^{b}
PBPL (x10⁹/L)	Day 0	6.52±0.36	7.45±0.30	6.62±0.62	6.80±0.32	6.80±0.25
	Day 7	9.98±0.41^{c}	8.95±0.50^{b}	7.05±0.06	10.90±0.23^{c}	7.77±0.12^{a}
	Day 14	8.60±0.37^{b}	8.85±0.25^{a}	7.00±0.52	11.10±0.25^{c}	8.30±0.29^{bx}
NEUT (%)	Day 0	57.00±1.72	62.00±2.01	60.75±2.87	59.75±1.09	58.25±1.90
	Day 7	63.50±1.41^{a}	57.00±1.63	60.25±2.01	65.50±0.73^{a}	56.00±1.57
	Day 14	61.75±1.39	60.75±1.20	58.00±1.26	62.00±1.21	64.50±1.61^{ay}
EOS (%)	Day 0	5.00±0.32	6.50±0.43	7.75±0.35	6.25±0.37	4.75±0.26
	Day 7	4.50±0.41	4.50±0.32^{a}	5.25±0.26^{a}	5.25±0.22	4.00±0.32
	Day 14	5.50±0.24	6.25±0.48	4.75±0.37^{bx}	6.00±0.41	5.50±0.27
PBML (x10⁹/L)	Day 0	3.98±0.28	3.42±0.34	3.02±0.24	3.52±0.27	4.00±0.31
	Day 7	4.72±0.12^{a}	5.60±0.51^{b}	3.70±0.33	4.50±0.23^{a}	5.40±0.33^{c}
	Day 14	4.20±0.28	4.35±0.32^{a}	4.10±0.22^{b}	5.20±0.33^{b}	4.10±0.25^{z}
LYM (%)	Day 0	30.50±1.59	25.00±1.72	25.25±1.95	27.25±1.02	31.25±1.83
	Day 7	25.25±0.85	30.25±1.42	27.75±1.78	24.00±0.68	30.75±1.42
	Day 14	25.00±0.91	26.00±1.11	29.50±1.21	26.50±1.15	24.50±1.58
MON (%)	Day 0	7.50±0.50	6.50±0.73	6.25±0.44	6.75±0.45	5.75±0.33
	Day 7	6.75±0.47	8.25±0.52^{a}	6.75±0.50	5.25±0.27	9.25±0.46^{c}
	Day 14	7.75±0.34	7.00±0.56	7.50±0.43^{a}	5.50±0.48	5.50±0.31^{z}
PCV (%)	Day 0	38.92±0.79	39.82±1.00	38.55±0.93	39.37±0.71	39.52±1.39
	Day 7	39.10±0.46	39.70±0.93	39.22±1.21	40.12±0.64	40.25±1.21
	Day 14	38.75±0.51	44.97±0.98^{b}	46.85±2.34^{a}	39.07±0.72	39.02±0.83
HGB (g/dl)	Day 0	12.40±0.66	14.15±0.45	13.52±0.42	13.27±0.47	14.22±0.59
	Day 7	12.77±0.54	14.50±0.35	13.72±0.64	13.12±0.54	14.32±0.55
	Day 14	13.22±0.41	14.55±025	15.02±0.49^{cx}	13.32±0.59	14.75±0.72
PLT (x10⁹/L)	Day 0	356.7±23.2	364.0±19.4	308.5±10.2	264.0±21.8	395.0±38.4
	Day 7	241.0±26.9^{b}	374.5±13.9	277.5±11.4	738.0±35.6^{c}	507.0±38.3^{b}
	Day 14	155.0±12.9^{cy}	470.2±36.1^{bx}	388.5±18.4^{ax}	553.0±23.3^{by}	379.0±30.3^{y}

Statistically significant difference at 0-7 and 0-14 days each group: ***a*** *P<0.05,* ***b*** *P<0.01,* ***c*** *P<0.001*
Statistically significant difference at 7-14 days each group: ***x*** *P<0.05,* ***y*** *P<0.01,* ***z*** *P<0.001*

concentrations in group 2 and 3 were not different AV compared to values obtained BV. Comparison between day 7 and 14 AV revealed that IgM concentration in group 1 decreased on day 14, whereas IgM and IgG concentration in group 5 increased on day 14. Serum IgA concentrations in all groups did not change throughout the study *(Table 3)*.

present study conflict with some previous studies with concern to peripheral WBCs [4,7,13], in which no change or rather a decrease in WBC count and differential leukocyte count (especially LYM) after vaccination by polyvalent vaccine were reported. On the other hand, Strasser et al.[6] reported that number of WBCs and band NEUTs increased significantly, presumably due to activation as

Table 2. *Effects on peripheral blood lymphocyte subsets of Aloe vera and β-Glucan administration alone or after polyvalent vaccine application in dogs (Mean±SEM)*

Tablo 2. *Köpeklerde tek başına ve karma aşı uygulaması sonrası Aloe vera ve β-Glucan uygulamalarının periferal kan lenfosit alt tipleri üzerindeki etkileri (Ortalama±Standart Hata)*

Parameters	DAYS	GROUP 1	GROUP2	GROUP 3	GROUP 4	GROUP 5
CD 3 T Lymphocyte (%)	Day 0	71.9±2.96	65.2±1.68	65.1±3.62	58.8±2.39	63.7±1.38
	Day 7	78.3±2.14^{b}	71.5±1.61^{a}	71.1±1.62^{a}	63.0±1.91^{a}	71.9±1.84^{c}
	Day 14	73.4±2.81^{y}	75.1±0.82^{bx}	72.6±2.78^{a}	73.6±1.69^{bx}	78.3±1.74^{cx}
CD 4 T Lymphocyte (%)	Day 0	42.7±1.92	33.4±1.63	33.0±1.91	33.0±1.50	34.7±1.60
	Day 7	44.5±1.98	41.6±2.15^{b}	41.8±1.49^{b}	36.8±1.79^{a}	38.1±0.98^{b}
	Day 14	42.0±1.69	46.0±1.82^{bx}	41.6±2.59^{b}	45.7±1.31^{cy}	43.7±1.56^{cx}
CD 8 T Lymphocyte (%)	Day 0	22.7±1.31	20.8±1.05	13.9±0.89	13.4±1.00	15.3±0.92
	Day 7	30.8±2.13^{b}	26.1±0.83^{b}	17.2±0.91^{b}	16.3±1.40^{a}	20.7±0.89^{c}
	Day 14	30.1±1.91^{b}	22.2±1.50^{a}	18.5±0.58^{c}	23.9±1.61^{cy}	21.2±1.17^{c}
B Lymphocyte (%)	Day 0	20.5±1.41	15.6±0.88	19.7±1.51	17.8±1.26	18.8±1.20
	Day 7	20.6±1.43	17.3±1.17	22.7±1.29	23.7±1.92^{b}	22.6±1.29^{a}
	Day 14	18.3±1.69	21.4±1.59^{ax}	25.2±2.09^{b}	24.4±2.40^{b}	20.6±1.21^{a}

Statistically significant difference at 0-7 and 0-14 days each group: ***a*** *P<0.05,* ***b*** *P<0.01,* ***c*** *P<0.001*
Statistically significant difference at 7-14 days each group: ***x*** *P<0.05,* ***y*** *P<0.01*

Table 3. *Effects on serum immunglobulin concentrations of Aloe vera and β-glucan administration alone or after polyvalent vaccine application in dogs (Mean±SEM)*

Tablo 3. *Köpeklerde tek başına ve karma aşı uygulaması sonrası Aloe vera ve β- glucan uygulamalarının serum immunglobulin konsantrasyonları üzerindeki etkileri (Ortalama±Standart Hata)*

Parameters	DAYS	GROUP 1	GROUP2	GROUP 3	GROUP 4	GROUP 5
Ig G (mg/dL)	Day 0	1386.2±9.60	1337.5±51.4	1371.0±14.0	1240.5±15.3	1268.0±16.5
	Day 7	1435.4±13.9	1322.6±40.5	1418.3±9.33	1482.0±15.5^{c}	1407.0±13.6^{b}
	Day 14	1517.3±23.7^{b}	1381.5±47.9^{a}	1465.5±9.80^{a}	1652.3±16.1^{c}	1623.1±18.7^{xc}
Ig M (mg/dL)	Day 0	174.6±3.34	181.5±9.43	189.5±3.57	169.0±5.79	170.1±5.33
	Day 7	226.2±5.34^{c}	184.7±5.37	202.2±3.54	213.0±5.35^{b}	205.2±3.93^{b}
	Day 14	182.0±4.14^{y}	188.0±4.10	190.0±1.68	209.1±5.79^{b}	215.0±4.71^{xc}
Ig A (mg/dL)	Day 0	58.87±5.19	60.77±6.87	62.87±7.71	62.30±5.51	59.32±7.65
	Day 7	60.47±4.04	59.82±6.96	61.02±6.13	61.82±6.61	60.92±7.24
	Day 14	60.05±6.29	61.57±7.24	62.30±6.77	62.05±6.59	61.27±7.68

Statistically significant difference at 0-7 and 0-14 days each group: ***a*** *P<0.05,* ***b*** *P<0.01,* ***c*** *P<0.001*
Statistically significant difference at 7-14 days each group: ***x*** *P<0.05,* ***y*** *P<0.01*

DISCUSSION

Immune modulating and/or restorating polyglucans have been used in humans and laboratory animals with many different applications [18,20,21,24,28,29,31,32,37,38]. The present study, however, is the first report of polyglucans commercially available *Aloe vera* and β-*Glucan* causing significant stimulation and/or restoration of some immune and haematological parameters following immunization with polyvalent vaccines in dogs.

In the present study, polyvalent vaccine application caused a significant increase in WBC, PBPL, PBML counts and NEUT ratios *(Table 1)*. The results obtained in the

was the case in our study where WBCs increased. This finding might originate from time shifts and alterations in WBC activation, trafficking and homing-in or even more, reflect the challange of the immune system by continious-and in our case- replication of virus in polyvalent vaccine as reported by Strasser et al.[6].

Dhein and Gorham [9] reported that vaccine-induced thrombocytopenia may occur within 1 to 2 weeks of vaccination. Furthermore, this situation may be mediated by a type II hypersensitivity reaction with vaccine antigens on the surface of the platelet serving as the target for antibody [9]. Similarly, in the present study, PLT counts decreased in the postvaccination period

(Table 1). These findings in our study were in parallel to that of Dhein and Gorham [9] findings, and the same reasons reported may play role.

Our investigation showed that while *Aloe vera* (group 2) enhanced/stimulated haematological parameters on both 7th and 14th days AT, this effects were obtained with β-*Glucan* (group 3) only on 14th day AV. But, EOS ratios was quite opposite and a decrease in EOS ratios was noted. Researchers demonstrated that following administration of *Aloe vera* and β-*Glucan* stimulate and/or enhance hemopoiesis, including formation of pluripotent hematopoietic stem cells, granulocyte macrophage colony-forming cells, and cells forming myeloid and erythroid colonies [33,34]. Therefore, generally determinated increasing haematological parameters (except EOS ratios) in our study may be attributed to the reasons explained in previous studies [33,34,36,41]. Furthermore, it is declared that administration of *Aloe vera* and β-*Glucan* can be highly stimulative on haematological parameters including WBC, NEUT, LYM, MON, PBPL, PBML, HGB, PLT [19,23-25,28,31,33,36]. This situation was previously reported by Talmadge et al.[42] where increased hematopoietic activity was associated with increased mRNA levels for hematopoietic cytokines. In our study, EOS ratios decreased after administration of *Aloe vera* and β-*Glucan*. Although there is no study with concern to the effects of *Aloe vera* on peripheral blood EOS ratios, β-*Glucan* known to cause decrease in peripheral blood EOS ratios [25]. This was in the line with the finding of this study regarding to EOS where EOS decrease has been associated with polyglucan use.

In the present study, oral administration of *Aloe vera* (group 4) and β-*Glucan* (group 5) in addition to polyvalent vaccine and in group 1 (only polyvalent vaccine applied) caused to increase the number of WBC, PBPL, PBML. Furthermore, *Aloe vera* and β-*Glucan* (group 2, 3, 4 and 5) caused increase PLT counts. In contrast, PLT counts decreased in group 1 *(Table 1).* The effects of *Aloe vera* and β-*Glucan* can be seen from above results. Because, in group 1 (only vaccine applied group) PLT counts decreased during post vaccination period. The situation explain the possible effect of *Aloe vera* and β-*Glucan* in preventing hemopoietic depletion or enhancing hemopoietic recovery [33,34,36,41]. Additionally, administration of *Aloe vera* following vaccination caused an increase in PBML counts at postvaccination period (7th and 14th days), whereas administration of β-*Glucan* following vaccination caused an increase in PBML counts and MON ratios at postvaccination period only on 7th day *(Table 1).* These findings suggested that both administration of *Aloe vera* and β-*Glucan* might stimulate LYM and/or MON ratios after vaccination. This situation may be explained by enhanced stimulation of WBC and differential leukocyte type by *Aloe vera* and β-*Glucan* as several researchers reported previously [19,24,25,33,34,36].

Several parameters reflecting cellular and humoral immun response after vaccination of dogs have been evaluated by some researchers [4,6-8,13]. In previous studies [8,13], lymphocyte subsets in polyvalent vaccines applied animals were evaluated. However, in these studies only CD4 and CD8 T lymphocytes in minimum ve maximum vaccination schedule groups examined [13], or lymphocyte subsets in young and old dogs were determined [8]. But, general profile after vaccination have not be evaluated to the best of our knowledge. For this reason, although lymphocyte subsets analysis after polyvalent vaccination which included CD3, CD4, CD8 T lymphocytes and B lymphocyte were performed in the present study *(Table 2),* discussion on lymphocyte subsets could not be made. In our study, cytokines, cell mediated immunity indicator parameters and plasma complement system activity were not evaluated.

It has been reported that *Aloe vera* and β-*Glucan* have diverse immunomodulatory and/or stimulatory activities *in vivo* as well as *in vitro*. These effects observed in both humoral and cellular immune reactions [18,21,25,28,29,31,34,37]. In previous experimental studies, increase and/or stimulation in CD3, CD4, CD8 T lymphocytes, B lymphocyte and IgG concentrations were related to administration of β-*Glucan* and *Aloe vera* [18,21,24-26,30,31,34,37,38]. Similarly, in the present sudy, CD3, CD4 and CD8 T lymphocytes ratios at 7th and 14th days AV, whereas B lymphocyte ratios and serum IgG concentrations at only 14th days AV increased in group 2 and 3 *(Table 2, 3).* Immunostimulating action of *Aloe vera* and β-*Glucan* reported to induce by potentiation of synthesis and release of several cytokines such as TNF-α, IFN-γ, IL-1, IL-2 and IL-6 [18,19,24,28-32,37]. Moreover, it has been shown that polyglucans significantly supported the formation of spesific and nonspesific antibodies [18,21,24,25,31,37]. For this reason, increase in serum IgG after application of only *Aloe vera* and β-*Glucan*, may be attributed to elevation in humoral immun response due to increase in both CD4 T lymphocyte and cytokines synthesis or release.

All lymphocyte subsets ratios and serum immunglobulin concentrations except for IgA increased in group 4 and 5 both on 7th day and 14th day AV in the present study *(Table 2, 3).* On the other hand, although CD3 (7th day) and CD8 T lymphocytes (7th and 14th days), serum IgG (14th day) and IgM (7th day) concentrations increased AV in group 1, CD4 T lymphocyte and B lymphocyte ratios did not change in the same period *(Table 2, 3).* Therefore, increases in both CD3 (especially on 14th day) and CD4 T lymphocytes ratios (7thand 14th days), and serum IgG and IgM concentrations on days 7 and 14

AV in group 4 and 5 may be attributed to modulatory or stimulatory effects which suggest the effects of *Aloe vera* and β-*Glucan* on both cellular and humoral immun response following vaccination. Similar observations on immune system by *Aloe vera* and β-*Glucan* have already been reported by authors [18,20,21,24-26,28,31,32,34].

CD8 T lymphocyte, a subclass of CD3 T lymphocyte, is completely related to cellular immune response, while CD4 T lymphocyte takes part in both cellular and humoral immune responses. Because, CD4 T lymphocytes are divided into Th1 and Th2 cells according to lymphokines that they release. Antigenic stimulation of Th1 cells induce T cell cytotoxicity and macrophage activation through release of cytokines like IL-2, IFN-γ and TNF-β and also in the stimulation of Th2 cells and production of B cells. Th2 cells plays role in the stimulation of IL-4, IL-5, IL-10 and IL-13 cytokines leading to production of B cells and antibodies [43-45]. Although Th1 and Th2 helper cells analysis were not performed in the present study, it is interesting to show the increase in CD4 T lymphocyte ratios after vaccination in group 4 and 5 *(Table 2)* which might be attributable to an increase in Th1 and Th2 helper cells, and this situation may reflect adequate cellular and humoral immune response associated with the use of *Aloe vera* and β-*Glucan* after vaccination. CD3 T lymphocyte ratios increased only on the 7th day AV in group 1 and CD4 T lymphocyte ratios did not change in the same period. But, the group received only *Aloe vera* (group 2), only β-*Glucan* (group 3), *Aloe vera* (group 4) and β-*Glucan* (group 5) in addition to vaccine caused an increase in both CD3 and CD4 T lymphocytes ratios on 7th and 14th days AV. On the other hand, B lymphocyte ratios increased in group 2 and 3 only on 14th day AV, whereas its ratios increased in group 4 and 5 on day 7th and 14th AV *(Table 2).* The present findings relating to lymphocyte subsets confirms the findings of authors where only *Aloe vera* and β-*Glucan* used [24,26,34]. Increased lymphocyte subsets such as CD3 and CD4 T lymphocytes in all groups except for group 1 *(Table 2)* might be explained by earlier findings [18,19,24,28-32,37] where synthesis and release of several cytokines such as TNF-α, IFN-γ, IL-1, IL-2 and IL-6 increased after *Aloe vera* and β-*Glucan* administration. But, CD8 T lymphocyte ratios increased in all groups on 7 and 14 days AV. Increased CD8 T lymphocyte ratios *(Table 2)* might be symbolise non spesific cellular immune response enhancer of *Aloe vera* and β-*Glucan*.

Aloe vera and β-*Glucan* are usually considered stimulators or modulators of humoral immune responses. Our results showed that serum IgM concentrations only on 7th day and IgG concentrations only 14th day AV elevated in group 1. Serum IgM and IgG concentrations in group 4 and 5 elevated both on 7th and 14th days AV. Moreover, serum IgG concentrations in group 2 and 3 increased only 14th day AV as similar to group 1, but serum IgM concentrations in the same groups did not change *(Table 3).* Serum IgG and IgM concentrations in post vaccination period in the present study (group 1, 4, 5) increased as reported by researchers [1,2,6,9]. On the other hand, the author's results of evaluating the effects of *Aloe vera* and β-*Glucan* on antibody response in laboratory animals showed that their potential to increase antibody response was present only when they used as an adjuvant [21,25,30,33,37,38,46] and/or significant increase in number of B lymphocytes forming both IgM and IgG antibodies [18,21,24,25,30,31]. In the present study, similar adjuvant effects [21,25,30,33,37,38,46] detected after *Aloe vera* and β-*Glucan* administration in group 4 and 5. But, serum IgM levels in group 2 and 3 did not change after *Aloe vera* and β-*Glucan* administration. Moreover, B lymphocyte ratios also increased in all groups except for group 1 *(Table 3).* This findings demonstrate that *Aloe vera* and β-*Glucan* may stimulate humoral immune response as reported by researchers [18,20,21,25,28,31,34].

In conclusion, this study demonstrated that oral administration of *Aloe vera* and β-*Glucan* affects various aspects of the canine immune system, including the effects on haematologic parameters, the composition of lymphocyte subsets, and serum immunglobulins. This findings demonstrate that *Aloe vera* and β-*Glucan* may stimulate both cellular and humoral immun responses after vaccination in dogs. The observed changes after administration of *Aloe vera* and β-*Glucan* following vaccination might occur in similar mechanisms. Moreover, our results suggest that the observed increase in platelet counts after administrations of *Aloe vera* and β-*Glucan* might have restorative effects on thrombocytopenia. However, since exact mechanisms of the stimulation of cell-mediated and humoral immunity related to *Aloe vera* and β-*Glucan* application after vaccination still remains unknown further investigations might be useful to clarify the stimulatory mechanisms through investigating Th1 and Th2 helper cells ratios, cellular and humoral immune system spesific cytokines.

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