



# Hematologic and serologic status of military working dogs given standard diet containing natural botanical supplements

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## ABSTRACT

The health of military working dogs (MWDs) deployed with Korean troops is of prime importance. The aim of our study was to investigate the hematologic and serologic status of Korean MWDs given natural botanical supplements. To do this, 11 natural botanicals were selected based on relevant references and combined to supplement MWDs. Throughout the 16-week experimental periods, there was no significant difference in body weights of individual dogs. The Hemoglobin (HGB), hematocrit (HCT), Mean Corpuscular Volume (MCV), and Mean Corpuscular Hemoglobin (MCH) values were slightly higher in the group given the supplement. On the other hand, the Mean Corpuscular Hemoglobin Concentration (MCHC) values were slightly lower. Changes in platelet, lymphocyte, and basophil counts were observed in the supplemented group. The median serum IL-6 level did not differ significantly between the supplemented and control groups. However, the mean serum C-reactive protein (CRP) value increased significantly from the start of supplementation to 8 weeks, and then decreased at 16 weeks. Taken together, our result suggests that the health condition of most MWDs supplemented with natural botanicals was gradually improved. Thus, this study may provide support for the development of a feed supplement for MWDs using natural botanicals.

## 1. Introduction

Hundreds of military working dogs (MWDs) are currently deployed with Korean troops, including the Republic of Korea Army, Air Force, and Navy. These highly trained animals provide various services such as explosive, mine, and drug detection; security; and rescue. All MWDs are maintained in excellent physical condition with routine obstacle course work and specialty training. Most of these dogs work 8–12 h a day several times a week. They are fed a standard diet, and each dog's weight is kept within standard limits established by the Korean Military Veterinary Services with the help of U.S. forces.

The health and well-being of these MWDs are of prime importance. Nevertheless, one of the categories of diseases that threaten their health is joint-related diseases such as osteoarthritis (OA) [1]. Specially, OA is a condition that causes pain, inflammation, and stiffness in many joints and commonly occurs as a consequence of joint dysplasia [2]. Even though the genetic background of select pedigreed breeds, excessive exercise, nutritional imbalances, chronic inflammation, and aging are also linked to the development of OA [2–4]. These inflammatory disorders are often treated using non-steroidal anti-inflammatory drugs

(NSAIDs) and disease-modifying anti-rheumatic drugs (DMARDs) [1,5,6]. However, these drugs for OA result in unwanted side effects and various studies are being conducted to overcome these problems.

An American study have suggested that 10–20% of all MWD retirements from service are due to degenerative joint disease [7]. A few years later, Smith et al. reported that restricting dogs' diets by 25% resulted in a significant delay in the onset of signs of hip arthritis [8]. This suggests a possibility for preventing disease by adjusting the dietary intake of MWDs. In this regard, botanical dietary factors are the subject of considerable interest in OA research [6,9].

As mentioned above, OA is primarily a pro-inflammatory disease. The role of inflammatory cytokines such as tumor necrosis factor (TNF)- $\alpha$ , interleukin (IL)-1 $\beta$ , IL-6 and chemokines; inflammatory enzymes such as cyclooxygenase (COX)-2, and matrix metalloproteinases (MMPs); and adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) in the pathogenesis of arthritis is well documented [10–12]. The inflammatory mediators linked to OA have been shown to be regulated by the transcription factor nuclear factor-kB (NF-kB) [11].

Nutritional management of inflammation is important in maintaining health in dogs. According to previous studies, some natural

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botanicals contain bioactive components with anti-inflammatory action and, when included in the diet, may contribute to a reduction in pro-inflammatory response. There are data to support the anti-inflammatory effects and the efficacy of such bioactive molecules from botanicals [for review see [6]. Among them, methyl sulfonyl methane (MSM) is used as a dietary supplement because of its potential to reduce arthritic pain [13]. The seeds of safflower (*Carthamus tinctorius* L.) are known to be effective against bone diseases such as fracture and osteoporosis [14]. *Cirsium japonicum* is a wild perennial herb native to Asia, including Korea, and has been used as an antihemorrhagic and antihypertensive agent [15]. Brown marine algae are traditionally used as a food and medicinal herb in East Asia [16,17]. Turmeric (*Curcuma longa*) is extensively used as a spice, food preservative, and coloring agent in Asia. It has been used in traditional medicine for various diseases, including rheumatism [18]. Curcumin (diferuloylmethane), the main yellow bioactive component of turmeric, has been shown to have various biological effects including anti-inflammatory action [18–20]. Extract of the roots and stems of *Acanthopanax senticosus* (Syn. *Eleutherococcus senticosus*) has been reported to have pharmacological action against rheumatism and allergies [21,22]. Glucosamine (Glu) is an amino-monosaccharide and the building block of proteoglycans, the base substances of articular cartilage [13]. Chondroitin sulfate (CS), a polymer of repeating disaccharide units (galactosamine sulfate and glucuronic acid), is the predominant component of articular cartilage [13]. The combination of Glu and CS has been shown to protect against chemically induced synovitis in dogs [23] and to stimulate cartilage metabolism, resulting in the inhibition of cartilage degradation [24,25]. Hyaluronan (HA) is a major component of both synovial fluid and articular cartilage [26,27]. OA treatment with intra-articular HA is an alternative treatment to NSAIDs [28]. A high dietary intake of the antioxidant nutrient vitamin C (ascorbic acid) has been suggested to slow osteoarthritis disease progression [29,30]. Finally, vitamin E is well known for its chondroprotective effects [31]. Studies have reported that the dietary supplementation with Vitamin E reduces symptoms of OA in human patients [32].

Currently, a lot of effort and high-cost are invested to make one good MWD in Korean troops. However, life expectancy as a MWD is relatively short. Thus, if we can extend the health of MWD by natural botanical supplements, they will become even more valuable, especially in a divided country like Korea. To date, there have been no studies regarding joint inflammation-related serum factors in Korean MWDs. Therefore, the objective of this study was to investigate the hematologic and serologic status of MWDs fed a diet with a supplemental mixture of natural botanicals. For this study, 11 species of natural botanicals were selected based on scientific references and mixed. Along with hematologic analyses, C-reactive protein (CRP) and IL-6 concentrations were analyzed in the serum of MWDs fed a diet supplemented with the botanical mixture.

## 2. Materials and methods

### 2.1. MWD sources

Military installations that submitted samples during the study period were Chuncheon Korea Army Base (CKAB), located in a mountainous area in the northwest; and Jinju Korea Air Force Base (JKAFB), located in the southern part of the Korean Peninsula. Age, breed, sex, and the date of sample collection were recorded.

### 2.2. Population characteristics

A total of 24 MWDs were included in the study – 9 MWDs were from the CKAB; another 15 were from the southern region JKAFB (Table 1). There were 11 MWDs in the 1- to 4-year-old age group, 11 in the 5- to 8-year-old age group, and 2 in the 9- to 12-year-old age group. There were 21 dogs in the 20–30 kg group and 3 dogs in the >30 kg group. The

breed distribution included 3 Labrador Retrievers and 21 German Shepherd Dogs. The sex distribution included 13 female dogs and 11 males. Fifteen German Shepherd MWDs (30–92 months old, mean body weight  $26.0 \pm 2.48$  kg) from the JKAB, and six German Shepherd (10–109 months old, mean body weight  $30.2 \pm 3.96$  kg) plus three Labrador Retriever MWDs (15–49 months old, mean body weight  $27.4 \pm 2.57$  kg) from the CKAB were randomly assigned to be supplemented.

### 2.3. Diet

Based on a literature survey, a mixture of natural botanicals containing MSM, safflower seed, thistle, seaweed fusiforme, turmeric, *Acanthopanax* root bark, Glu HCl, CS, Hyaluronic acid, and Vitamin C/E was produced, and then given to MWDs as a dietary supplement; Individual botanicals are well-known in oriental medicine to be beneficial to human health. Assigned two groups of MWD were supplemented daily with 500 mg capsulated formulation diet for 0–16 weeks (Table 2). The basal diet met or exceeded the requirements for all essential nutrients (data not shown). Body weight was recorded at 0 and 16 weeks. The research protocol was approved by the Institutional Animal Care and Use Committee of Konkuk University in Seoul, Korea. One dog (No 15 in Table 1) was died of acute pneumonia in two months after starting the experiment.

### 2.4. Blood sample collection

Whole-blood samples were collected from MWDs by a licensed veterinary officer at their home Korean military locations. Blood (5 mL) was collected from the cephalic vein of the foreleg into Z Serum Sep Clot Activator tube (Greiner Bio-One, Kremsmünster, Austria) and K2-EDTA whole blood collection tube (LP ITALIANA, Milano, Italy) at 0, 8, and 16 weeks, and then sent to the KNOTUS institute (Guri, Korea) for hematologic analysis. Serum was obtained within 2 h of blood sample collection via centrifugation at 2400 g for 5 min; serum was harvested, transferred to cryovials, and immediately frozen ( $-20^{\circ}\text{C}$ ) and stored until analysis.

### 2.5. Hematologic analysis

Blood analysis was performed on each sample by licensed medical technologists using an automated hematology analyzer (ABX MICROS 60, France) in the laboratory on the day of blood collection. Only samples without blood clots were analyzed. Hematological parameters included leukocyte subpopulations profile comprising total white blood cells (WBC) count and erythrocyte profile consisting of red blood cells (RBC) count; differential leukocyte counts (lymphocytes, monocytes, neutrophils, eosinophils, and basophils), and hemoglobin (HGB), hematocrit (HCT), platelet, mean cell volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC).

### 2.6. Enzyme-linked immunosorbent assay (ELISA)

CRP and IL-6 are prognostic biomarkers in dog osteoarthritis [33,34]. The analyses of canine serum CRP and IL-6 were performed with commercially available canine-specific ELISA kits. CRP (PTX1) Dog ELISA Kits were purchased from Abcam (Cambridge, MA, USA). Canine IL-6 Quantikine ELISA Kits were purchased from R&D Systems (Minneapolis, MN, USA). All serum samples were analyzed in duplicate according to the manufacturer's instructions. Serum levels of CRP and IL-6 were determined by sandwich ELISA using the combination of specific canine monoclonal and polyclonal antibodies.

**Table 1**

The summarizing details of the individual MWD.

No.	Sex	Age (month)	Breed	Color	Weight (kg)	Military base	Formulation
1	M	65	GSD <sup>a</sup>	Wolf-gray	24.5	JKAFB <sup>c</sup>	1
2	M	85	GSD	Black and tan	26.8	JKAFB	1
3	M	40	GSD	Black	28.4	JKAFB	1
4	M	53	GSD	Wolf-gray	22.4	JKAFB	1
5	F	89	GSD	Wolf-gray	27.6	JKAFB	1
6	F	85	GSD	Wolf-gray	22.1	JKAFB	1
7	F	92	GSD	Black and tan	28.9	JKAFB	1
8	F	91	GSD	Wolf-gray	27.0	JKAFB	2
9	F	65	GSD	Wolf-gray	24.7	JKAFB	2
10	F	68	GSD	Wolf-gray	28.7	JKAFB	2
11	F	64	GSD	Wolf-gray	27.2	JKAFB	2
12	F	39	GSD	Wolf-gray	27.0	JKAFB	2
13	F	42	GSD	Wolf-gray	25.1	JKAFB	2
14	F	33	GSD	Black and tan	28.8	JKAFB	2
15	F	30	GSD	Black and tan	21.5	JKAFB	2
16	M	15	LR <sup>b</sup>	Yellow	28.5	CKAB <sup>d</sup>	1
17	F	10	GSD	Black and tan	27.9	CKAB	1
18	M	61	GSD	Black and tan	30.0	CKAB	1
19	M	88	GSD	Black and tan	33.7	CKAB	1
20	M	109	GSD	Black and tan	28.1	CKAB	1
21	M	50	LR	Yellow	29.3	CKAB	2
22	M	50	LR	Yellow	24.5	CKAB	2
23	F	10	GSD	Black and tan	25.7	CKAB	2
24	M	110	GSD	Black and tan	36.2	CKAB	2

<sup>a</sup> GSD: German shepherd dog.<sup>b</sup> LR: Labrador Retriever.<sup>c</sup> JKAFB: Jinju Korea Air Force Base.<sup>d</sup> CKAB: Chuncheon Korea Army Base.

### 2.7. Statistical analysis

Data were analyzed using PROC MIXED of SAS package program (2002–2003, release. 9.3 version, SAS inc., Cary, NC, U.S.A.) with a complete randomized design. Model was,

$$Y_{ij} = \mu + T_i + E_{ij}$$

where  $\mu$  was an average value,  $T_i$  was treatment value, and  $E_{ij}$  was the error value. The experimental unit of this study is a military working dog and fixed effect was time (week) effect. The pair-wise comparison among treatments was conducted using CONTRAST statement. Statistical difference was accepted at  $p$  value of less than 0.05. All means are presented as least square means.

### 3. Results and discussion

Based on a literature survey, two types of different formulation of natural botanical supplements containing MSM, safflower seed, thistle, seaweed fusiforme, turmeric, *Acanthopanax* root bark, Glu HCl, CS, Hyaluronic acid, and Vitamin C/E were prepared, and then given to MWDs as a dietary supplement. Individual botanicals are well-known in oriental medicine to be beneficial to human health. Table 2 shows detailed information of the supplement given to two groups of MWDs; Formulation one was used in the first group and formulation two was used in the second group. The overall compositions of the two formulations differ only slightly. A statistical analysis of results was done for results from formulation one and from formulation two. There were not statistically significant differences in the results from the different formulations. Therefore, the results from the two formulations were combined for presentation. After supplementation, the body weight of individual MWDs was not affected within the experimental period, suggesting that there was no direct relationship between natural botanicals and obesity.

The hemogram analysis revealed some significant changes within and between the groups of MWDs from the start to the end of the study, though most values remained within the reference ranges (Table 3). The

hemoglobin (HGB) and hematocrit (HCT) values were slightly increased in the MWD group (HGB: 0 vs 8,  $P = 0.006$ ; HCT: 0 vs 8,  $< 0.001$ , 0 vs 16,  $P = 0.008$ ) given the supplement for 8 or 16 weeks. The platelet counts were significantly increased in this group (0 vs 16,  $P = 0.024$ ) after they received the botanical supplement. The Mean Corpuscular Volume (MCV) and Mean Corpuscular Hemoglobin (MCH) values were slightly increased in the supplemented group (MCV: 0 vs 8,  $P < 0.001$ , 0 vs 16,  $P < 0.001$ ; MCH: 0 vs 8,  $P = 0.006$ , 0 vs 16,  $P = 0.002$ ). On the other hand, the Mean Corpuscular Hemoglobin Concentration (MCHC) values were slightly decreased in the supplemented group (0 vs 8,  $P = 0.008$ , 0 vs 16,  $P = 0.011$ ). An increase in lymphocyte counts in the supplemented group (0 vs 8,  $p = 0.002$ ) was observed. Neutrophil counts were slightly increased in that group during the same period (0 vs 8,  $< 0.001$ ). Basophil counts were significantly higher in the supplemented group (0 vs 8,  $P = 0.040$ , 0 vs 16,  $< 0.001$ ).

Leukocytosis is a typical inflammatory process that temporarily increases immature neutrophils and is considered a sign of acute infection [35]. Through hematologic analysis, we observed a reduction in neutrophil percentage by the end of the experiment, suggesting that the acute innate immune response is likely to be gradually stabilized by supplementation with these natural botanicals. In general, basophils are very rare in healthy dogs. When basophil numbers are higher than normal in dogs, it is often in conjunction with an increase in the number of eosinophils, which is associated with allergies [36]. However, we could not observe a change in eosinophil percentage during the experiment. Basophils are one of granulocytes containing histamines, a compound intimately involved in allergic and asthmatic reactions, in the cytoplasm. Therefore, further study is needed to determine what substance or substances caused the increase in the number of basophils in the MWDs supplemented with natural botanicals. From another point of view, it is presumed that increased basophil numbers may be due to internal parasites or fleas in dogs [37]. However, this is very unlikely in MWD because MWDs in CKAB and JKAFB are regularly prescribed internal and external parasites medications. Supplementation with natural botanicals resulted in increased circulating platelet counts by the end of the experiment. Several studies have reported that acute exercise results in a transient increase in platelet count caused by

**Table 2**  
Materials and formulations of supplements fed to MWD.

Material	Botanical name	Main component	Purity (%)	Source	Formulation 1		Formulation 2		Reference <sup>b</sup>
					Content (%)	Daily intake (mg/day)	Content (%)	Daily intake (mg/day)	
Pine organic sulfur/MSM	<i>Pinus densiflora</i>	Methyl sulfonyl methane	98	YBSH co., Korea	20.0	100	25.0	125	Arafa et al. [13]
Safflower seed	<i>Carthamus tinctorius</i>	Linolenic acid	-	KTM <sup>a</sup>	10.0	50	9.0	45	Nordstrom et al. [14]
Thistle	<i>Cirsium japonicum</i>	Silymarin	-	KTM	10.0	50	9.0	45	Dixit et al. [15]
Seaweed fusiforme	<i>Hizikia fusiformis</i>	Fucoidan	-	KTM	10.0	50	9.0	45	Lee et al. [16]
Turmeric	<i>Curcuma longa</i>	Curcumin	-	KTM	10.0	50	9.0	45	Chattopadhyay et al. [18]
The root bark of Acanthopanax	<i>Acanthopanax</i>	Coumarin	-	KTM	10.0	50	9.0	45	Hemshekhhar et al. [22]
-	-	Glucosamin hydrochloride	99	Hwail co., Korea	12.8	64	12.8	64	Arafa et al. [13]
-	-	Chondroitin sulfate	99	Hwail co., Korea	5.0	25	5.0	25	Arafa et al. [13]
-	-	Hyaluronic acid	99	Humedix co., Korea	2.0	10	2.0	10	Uitterlinden et al. [26]
-	-	Vitamin C	20	Dalim co., Korea	10.0	50	9.4	47	Perego and Wilder [29]
-	-	Vitamin E	100	Dalim co., Korea	0.2	1	0.8	4	Aslan et al. [31]
Total					100.0	500.0	100.0	500.0	

<sup>a</sup> KTM: Kyungdong traditional market, Korea.

<sup>b</sup> The detailed information of individual component is referred.

**Table 3**  
Blood hemogram results at start (baseline), 8 and 16 weeks in MWDs given natural botanical supplements.

	Week			SEM <sup>1</sup>	P value	
	0	8	16		0 vs 8 <sup>A</sup>	0 vs 16 <sup>B</sup>
CRP (µg/mL)	12.66	21.51	8.30	2.972	0.050	0.337
IL-6 (pg/ml)	31.50	31.34	26.95	8.338	0.990	0.718
WBC (10 <sup>3</sup> /mm <sup>3</sup> )	10.19	9.54	11.50	0.604	0.479	0.160
RBC (10 <sup>6</sup> /mm <sup>3</sup> )	7.14	7.06	6.83	0.151	0.700	0.158
HGB (g/dl)	15.82	17.00	16.37	0.284	0.006**	0.185
HCT (%)	42.34	50.38	47.20	1.155	< 0.001***	0.008**
Platelet (10 <sup>3</sup> /mm <sup>3</sup> )	229.79	193.76	327.91	26.271	0.400	0.024*
MCV (µm <sup>3</sup> )	59.25	71.46	71.40	0.558	< 0.001***	< 0.001***
MCH (pg)	22.17	24.12	24.33	0.379	0.006**	0.002**
MCHC (g/dl)	36.50	33.81	33.93	0.580	0.008**	0.011*
Lymphocyte (%)	19.92	11.42	22.97	1.586	0.002**	0.238
Monocyte (%)	6.63	41.20	4.69	12.934	0.270	0.951
Neutrophil (%)	66.00	78.14	62.80	2.027	< 0.001***	0.314
Eosinophil (%)	7.46	8.92	8.91	1.079	0.372	0.374
Basophil (%)	0.00	0.23	0.54	0.064	0.040*	< 0.001***

SEM<sup>1</sup>: Standard error mean. 0 vs 8<sup>A</sup>; Comparisons between the 0 week and 8 weeks. 0 vs 16<sup>B</sup>; Comparisons between the 0 week and 16 weeks.

\* p < 0.05.

\*\* p < 0.01.

\*\*\* p < 0.001.

hemoconcentration and platelet release from the liver, lungs, and the spleen [38,39], and that the subsequent formation of platelet-leukocyte aggregates (PLAs) is, in part, due to increased platelet P-selectin is detected and increased [38]. Thus, an increase in platelets due to strenuous exercise may lead to the secretion of various inflammatory mediators involved in both innate and adaptive immune responses.

CRP levels in healthy dogs (mean 5.5 ± 1.8 µg/mL) and in dogs with OA (mean 9.3 ± 1.2 µg/mL) have been reported in previous studies [40,41]. In our study, the mean serum CRP concentration in MWDs increased significantly from the start of the experiment (mean 12.66 ± 2.015 µg/mL; P = 0.0006) to 8 weeks (mean 21.51 ± 4.130 µg/mL; P = 0.051). Then, it was decreased at 16 weeks (mean 8.30 ± 2.770 µg/mL; P = 0.337) (Table 3). It has been established that increased CRP concentrations in the blood are strongly associated with acute inflammation in humans [42,43] and dogs [44,45]. It is still controversial as to whether the CRP level is a more reliable marker than neutrophil count to quantify the severity of infection in acute disease [44,46]. However, it has been stated that in patients with clinical evidence of inflammation, CRP elevation can be of diagnostic value [46]. Nakamura et al. showed that dogs with active polyarthritis had higher CRP values than dogs with inactive disease [47]. However, because in acute inflammatory response activated neutrophils are the first immune cells to migrate to the inflammation site, both CRP and neutrophils may be considered biomarkers. Our result shows that serum CRP level and neutrophil percentage were both significantly lower following botanical supplementation.

Serum IL-6 levels have been reported for healthy dogs (8.0–11.4 pg/mL, median 9.2 pg/mL) and for dogs with OA (10.2–26.5 pg/mL, median 15 pg/mL) were reported [34]. In this study, the median serum IL-6 concentration of MWDs was high initially (mean 31.50 ± 11.865 pg/mL; P < 0.01), at 8 weeks (mean 31.34 ± 8.722 pg/mL; P = 0.990), and at 16 weeks (mean 26.95 ± 4.427 pg/mL; P = 0.718) (Table 3). There were no significant differences in serum IL-6 levels between the two groups. Cytokines play an important role as regulators of immune response [48], and thus cytokine profiles contribute to the effects of immunity level on many diseases [49]. In previous studies, IL-6 was shown to play a pro- or anti-inflammatory role in the pathophysiology of OA and, in some studies, was classed as a diagnostic and prognostic biomarker [50]. However, in our study, no change in serum IL-6 levels was observed.



Taken together, our results suggest that the health of most of MWDs supplemented with the mixture of 11 natural botanicals was gradually improved. Thus, our results may be used as data in developing feed using natural botanicals as supplements for MWDs. However, further research is needed to fully evaluate the effects of natural botanicals on MWDs.

### Conflicts of interest

All the authors declare that there are no conflicts of interest.

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