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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)-α, interleukin (IL)-1β, 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-κB) [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 ± 2.48 kg) from the JKAB, and six German Shepherd (10–109 months old, mean body weight 30.2 ± 3.96 kg) plus three Labrador Retriever MWDs (15–49 months old, mean body weight 27.4 ± 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 °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.