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Non-Controlled Clinical Efficacy Study Following Brain Six Complex Extract© Administration in Dogs with Cognitive Dysfunction Syndrome

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Abstract The incidence of canine cognitive dysfunction syndrome (CCDS), a prominent geriatric disease, is increasing because of the extended lifespan of companion animals. Various complementary therapies have been proposed for the management of CCDS. This study evaluated the clinical efficacy of the Brain Six Complex Extract© in dogs with cognitive dysfunction syndrome (CDS). Fifteen dogs with CDS were included, and four to five drops of Brain Six Complex Extract©, composed of herbal extracts, were applied around the dorsal neck of all dogs twice daily for 1-3 months. Clinical efficacy was evaluated using the CCDS scale, and serum β-amyloid oligomer concentrations were measured before and after administration of the extract. The CCDS scale score significantly decreased after administration in dogs with CDS ($p = 0.0313$), compared to pre-administration levels. Although the serum β-amyloid oligomer concentration decreased after administration, the change was not statistically significant ($p > 0.05$). A notable decrease was observed between pre- and post-administration in dogs with β-amyloid levels >300 pg/mL ($p = 0.0313$). The laboratory results showed no remarkable adverse effects of the extract. This study suggests that Brain Six Complex Extract© extract could be an adjunctive treatment for dogs with CDS.

Key words canine cognitive dysfunction syndrome, dogs, herbal extract, aromatherapy, β-amyloid.

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Introduction

Canine cognitive dysfunction syndrome (CCDS), also known as canine cognitive dysfunction (CCD), canine dementia, or cognitive dysfunction syndrome (CDS) in dogs, is a neurodegenerative condition affecting aged dogs. This syndrome is characterized by behavioral alterations, including diminished learning and memory capacities, disorientation, changes in the sleep-wake cycle, and house soiling (10). CCDS is similar to Alzheimer's disease (AD) in humans and is characterized by neuropathological changes, such as significant cortical atrophy, cerebral amyloid angiopathy, and ventricular enlargement (11). Moreover, oxidative stress plays a significant role in the pathogenesis of neurodegenerative diseases, including AD and CCDS (5).

The prevalence of CCDS is up to 60% in dogs, predominantly >11 years; however, the actual diagnosis rate remains quite low, at approximately 2% (5,12,14). Species-specific differences in CCDS have not been identified (12). The major clinical signs of CCDS include apparent confusion, anxiety, disturbance of the sleep/wake cycle, and decreased interactions between pets and their owners (3).

With the extended lifespan of companion animals, the importance of CCDS, a representative geriatric disease, is increasing. However, accurate treatments remained elusive until recently (14). Various therapeutic approaches have been reported to enhance cognitive abilities and improve quality of life (3). Several dietary interventions and supplements, including with CCDS such as omega-3-polyunsaturated fatty acids, medium-chain triglycerides, and S-adenosylmethionine, are known to help with CCDS (3). Furthermore, environmental enrichment has been demonstrated to enhance behavioral health and the overall quality of life in companion animals, along with improving cognitive function (8).

Currently, aromatherapy is a non-pharmacological complementary intervention aimed at ameliorating the symptoms of dementia in humans (1). One study reported the positive impact of aroma-infused bath salts on sleep-related aspects of AD (7). Moreover, the potential of aromatherapy extends to various geriatric syndromes, such as aspiration pneumonia, dyspnea, and cognitive decline in humans (4). However, only a limited number of studies have explored the clinical effects of aromatherapy on CCDS in veterinary medicine. This study aimed to assess the clinical efficacy of the Brain Six Complex Extract© in dogs with CDS based on the CCDS questionnaire and measurement of β-amyloid levels.

Materials and Methods

Test compound

The basic components of the Brain Six Complex Extract© consisted of extracts from the Iris, Wonji root, Bokryeong, Angelica root, Ginseng, and Jujube in addition to purified water, ethanol, noni fruit extract, sodium benzoate, and citric acid. The extract was characterized by an oil-based aroma derived from these constituents.

Animals and study design

This study was designed to assess the efficacy of the Brain Six Complex Extract© as a supplement in older dogs with CDS. Dogs diagnosed with CCDS were recruited and enrolled with the owners' consent. To diagnose cognitive dysfunction, a comprehensive medical history review, physical examination, and neurological assessment were conducted. All the procedures were approved by the Institutional Animal Care and Use Committee (PTB-2021-IACUC-009-A). Four to five drops of Brain Six Complex Extract© were applied around the dorsal neck of all dogs twice daily for at least 1 month and up to 3 months. Dogs with diseases other than CCDS that could cause neurological problems under the judgment of a veterinarian, such as kidney or liver disease, and those exhibiting behavioral abnormalities other than CCDS, were excluded from the study. The efficacy of the extract was evaluated by measuring the CCDS scores and serum β-amyloid levels before and after administration. The safety of the extract was examined by laboratory evaluations, including complete blood count (CBC) and serum chemistry, both before and after administration.

Laboratory examination

Laboratory examinations included CBC (Celltac alpha, Nihon Kohden, Japan) analysis and serum chemistry (DRI-CHEM NX 500I Clinical Chemistry analyzer, Fujifilm, Europe) profiles in all dogs before and after the administration. The CBC evaluation included white blood cell count, red blood cell count, hemoglobin level, hematocrit, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, and platelet count. The serum chemistry profiles included aspartate aminotransferase, alanine transaminase, alkaline phosphatase, blood urea nitrogen, creatinine, sodium, potassium, and chloride levels.

CCDS scale

The owners assessed the CCDS scores of all 15 dogs using the previously established "Senior Canine Behavior Questionnaire" method (11). The scoring scale applied to the CCDS

signs in the questionnaire comprised four levels (0, none or no change; 1 = mild; 2 = moderate; 3 = severe).

Serum β-amyloid oligomer concentrations

Serum β-amyloid oligomer concentration was analyzed using photooxidation-induced fluorescence amplification (ABSOL HS; Absology, Korea) before and after administration of the extract. Photooxidation-induced fluorescence amplification was performed according to manufacturer's instructions. Briefly, the serum samples and kit components were equilibrated to room temperature prior to use. Subsequently, 110 μL of enhancer solution, 110 μL of stable peroxide solution, and 2.2 μL of ADHP (10-Acetyl-3,7-dihydroxyphenoxazine) concentration were added to the microcentrifuge tubes. The tubes were then vortexed and centrifuged. The mixed solution or sample was added to appropriate wells of the kit. The kit was inserted into the test equipment (ABSOL HS). Following the completion of the test, the test results were measured after approximately 20 min.

Statistical analysis

All continuous data are expressed as mean ± standard deviation. All statistical analyses were performed using SPSS version 20 (IBM Corp., Armonk, NY, USA). The normality of the data was assessed using the Kolmogorov-Smirnov test. Paired t-tests were used to compare the differences in CCDS scores before and after application of the extract in all dogs. Alterations in β-amyloid oligomer concentrations before and after application of the extract were statistically analyzed using Wilcoxon matched-pair signed-rank tests. Statistical significance was set at $p < 0.05$.

Results

Baseline characteristics

A total of 15 dogs were enrolled in the study, with a mean age of 13.21 ± 2.91 years. The breeds included were Maltese (n = 5), Poodle (n = 3), Yorkshire Terrier (n = 2), Shih Tzu (n = 1), Pekingese (n = 1), Golden Retriever (n = 1), Chow (n = 1), and mixed breeds (n = 1). Of these, seven females (all spayed) and eight males (five castrated and three sexually intact) were included. The mean body weight of all dogs was 7.69 ± 7.76 kg.

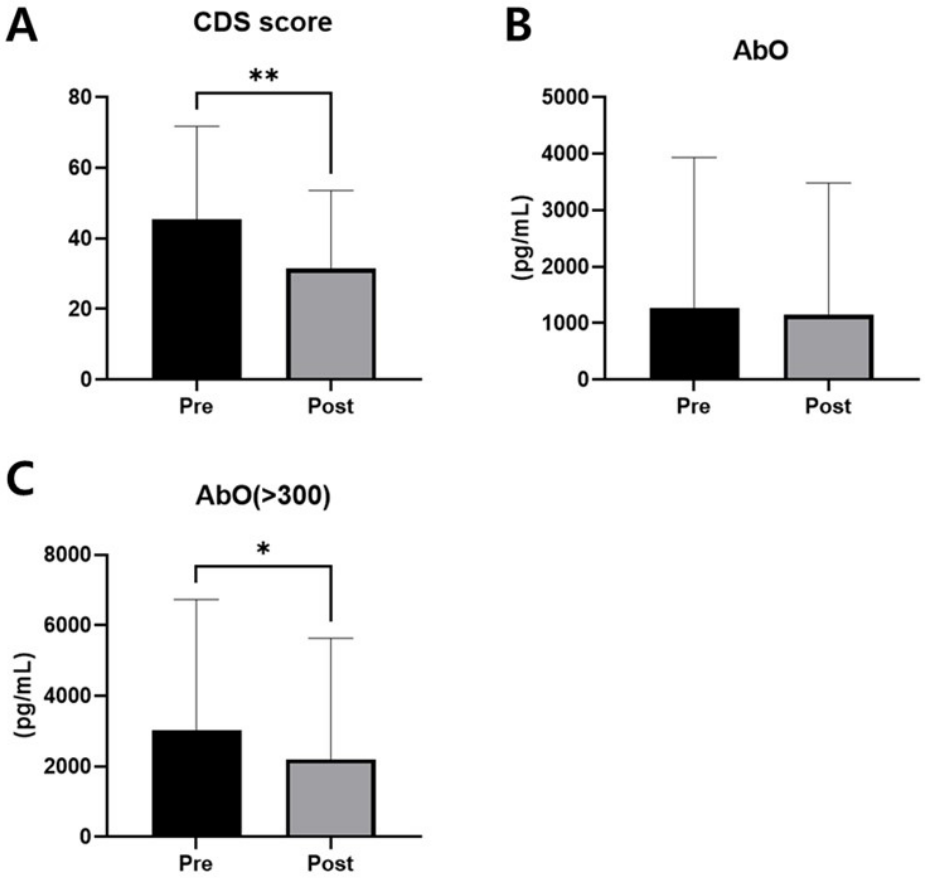

Fig. 1. Canine cognitive dysfunction syndrome scores and serum β-amyloid levels of dogs before and after administration of Brain Six Complex Extract©. AbO, β-amyloid oligomer; CDS, cognitive dysfunction syndrome. *$p < 0.05$, **$p < 0.01$.

Evaluation of clinical efficacy

To evaluate the efficacy of the extract for the CCDS, CCDS scores were compared before and after administration in dogs. CCDS scale was significantly decreased from pre-administration (45.40 ± 26.34) to post-administration (31.47 ± 22.09; p = 0.0077; Fig. 1A).

Furthermore, serum β-amyloid oligomer concentrations related to the CCDS were evaluated before and after extract administration. Following administration, serum β-amyloid oligomer concentration decreased (1,146.35 ± 2,333.86 pg/mL) compared to pre-administration levels (1,270.13 ± 2,664.84 pg/mL), although this change was not statistically significant (Fig. 1B). A significant decrease was observed when comparing the levels before (3,022.46 ± 3,705.29 pg/mL) and after administration (2,197.86 ± 3,428.93 pg/mL) in dogs with a β-amyloid oligomer level >300 pg/mL before administration (p = 0.0313; Fig. 1C).

Safety evaluation

Laboratory analyses were conducted on 15 dogs to evaluate the safety of the extract before and after administration. Laboratory examinations revealed no significant abnormalities in any dogs in the CBC tests before and after administration. Similarly, serum chemistry profiles revealed no remarkable findings before or after administration (Table 1).

Discussion

This study showed that the administration of an oil-based aroma extract improved the CCDS score in dogs with CDS. In humans, aromatherapy is recognized as a potential preventive and therapeutic approach for neurodegenerative diseases such as AD (9). Although the precise mechanism remains unclear, it is conjectured that olfactory stimulation activates the limbic system, including the hippocampus and amygdala (7). Dogs are known to respond positively to specific scents, although the exact underlying mechanisms are not fully understood (16). Nonetheless, the oil-based extract used in this study may contribute to ameliorating the clinical signs of CCDS.

Currently, there is no cure for CCDS; various nutritional and supplementary methods have been suggested (3). Nutraceuticals and medications, such as melatonin, valerian root, and dog-appeasing pheromones have been suggested to alleviate the clinical signs of CCDS (3). In this study, it has been demonstrated that aromatherapy could be used as an adjuvant treatment for CCDS. Further research is warranted to investigate various substances and methodologies that can be applied to treat CCDS.

β-amyloid deposition is a representative pathological change in AD and CCDS (3,9). β-amyloid peptides are 38 to 43 amino acids in length, which are enzymatically cleaved from amyloid precursor protein, and β-amyloid-related biomarkers are considered effective indicators for the early stage

Table 1. Complete blood counts and serum chemistry results of 15 dogs before and after administration of the Brain Six Complex Extract©

Tests		Before administration	After administration
CBC	WBC ($\times 10^3/\mu L$)	9.80 ± 5.16	10.83 ± 6.65
	RBC ($\times 10^6/\mu L$)	6.39 ± 1.12	6.60 ± 0.84
	Hb (gm/dL)	15.05 ± 3.18	15.15 ± 2.07
	PCV (%)	42.89 ± 8.03	43.55 ± 6.68
	MCV (fL)	67.11 ± 4.41	65.91 ± 4.97
	MCH (pg)	23.45 ± 1.68	22.97 ± 1.63
	MCHC (%)	35.01 ± 2.74	34.95 ± 2.60
	PLT ($\times 10^3/\mu L$)	431.13 ± 164.61	355.54 ± 176.87
Serum chemistry	AST (U/L)	39.69 ± 29.11	28.86 ± 6.76
	ALT (U/L)	68.40 ± 47.75	72.20 ± 40.01
	ALP (U/L)	332.20 ± 396.76	312.07 ± 445.17
	BUN (mg/dL)	23.19 ± 9.41	25.81 ± 12.30
	Cre (mg/dL)	0.81 ± 0.21	0.84 ± 0.19
	Na (mmol/L)	147.70 ± 6.86	149.06 ± 4.86
	K (mmol/L)	4.72 ± 0.75	4.93 ± 0.58
	Cl (mmol/L)	109.95 ± 6.32	111.75 ± 6.89

CBC, complete blood counts; WBC, white blood cell; RBC, red blood cell; Hb, hemoglobin; PCV, packed cell volume; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PLT, platelet; AST, aspartate transaminase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; BUN, blood urea nitrogen; Cre, creatinine; Na, sodium; K, potassium; Cl, chloride.

of AD (14). Moreover, therapies targeting β-amyloids, such as β-secretase and γ-secretase inhibitors, treat human AD (13,17). Senile plaques formed by extracellular β-amyloid and intracellular neurofibrillary tangles contribute to extensive synaptic loss and neuronal degeneration, leading to memory impairment, cognitive decline, and behavioral dysfunction in AD (13). β-amyloid naturally accumulates in the brain, and both dogs and humans have the same sequence of β-amyloid (5). β-amyloid oligomers are neurotoxic soluble aggregates from amyloid monomers in conditions of reduced clearance of the brain due to aging or genetic causes, which induce tau hyperphosphorylation and aggregation in neurofibrillary tangles, neuronal and mitochondrial damage, cell membrane destruction, and homeostasis dysregulation of calcium ions (6,15). β-amyloid oligomers act as upstream triggers in the early pathogenesis of AD and later accumulate into insoluble beta sheets constituting amyloid fibrils and plaques (15). β-amyloid oligomers consist of 2-12 monomers and the ratio of soluble β-amyloid 42 to β-amyloid 40 could be used as a diagnostic biomarker of AD (2). One study reported that total plasma β-amyloid levels could be used as a preselection tool for aged dogs with cognitive dysfunction (14). Taken together, these results suggest that the Brain Six Complex Extract© extract may exert beneficial effects by lowering β-amyloid concentration in dogs with a β-amyloid oligomer level >300 pg/mL and facilitating clinical improvement in the management of CCDS.

This study had several limitations. First, the number of dogs was relatively small, and the study lacked a control group. In addition, the mechanism underlying the application of the extract and its role in β-amyloid oligomer reduction could not be identified. Despite the limitations of this study, no adverse effects were observed after the administration of the extract. Therefore, further long-term, large-scale studies are necessary to identify the clinical efficacy and safety of the Brain Six Complex Extract© extracts. Further research is required to assess the mechanisms underlying the potential therapeutic action of the extract in managing CCDS.

Conclusions

This study describes the clinical efficacy of an oil-based extract as a complementary non-pharmacological therapy for CCDS. Notably, no significant adverse effects were noted following the administration of the Brain Six Complex Extract© Extract. Moreover, evaluating the CCDS scores and serum β-amyloid oligomer levels demonstrated favorable clinical outcomes. Therefore, the Brain Six Complex Extract© extract has potential as an adjunctive treatment for dogs with CDS.

Conflicts of Interest

The authors have no conflicting interests.

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