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Evaluation of the Effects of Ribavirin and Proanthocyanidin on the Clinical Outcome, Hematological and Biochemical Parameters, and Viral Shedding in Canine Distemper

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Antiviral agents, Canine distemper virus, Proanthocyanidins, Ribavirin

Abstract: This study investigated the effects of ribavirin, proanthocyanidin, and ribavirin-proanthocyanidin in dogs naturally infected with canine distemper virus (CDV). Five groups were created, each with six dogs aged 2-6 months: one healthy control group and four patient groups. For ten days, the A group received classical treatment (CT) [fluid treatment and antibiotic], the A+R group received CT + ribavirin, the A+P group received CT + proanthocyanidin, A+R+P group received CT + ribavirin-proanthocyanidin. On days T0, T3, T7, and T10, hematological, biochemical, and clinical scores were done. These days and the fifth and tenth post-treatment days were also screened for CDV. Clinical improvement was best in the A+P, A+R, A, and A+R+P groups, respectively. The A+R group had fewer leucocytes, neutrophils, and monocytes ($P<0.05$). CK and CK-MB activity were significantly higher only in the A group ($P<0.01$), and decreased only in the A+R group as therapy progressed. Creatinine values were high in A+P and A+R groups ($P<0.01$). The lowest calcium value was in the A+R group ($P<0.01$). The least CDV presence was detected in the A+R group regarding viral shedding. As a result, using ribavirin or proanthocyanidin helped to lessen the severity of clinical findings, increase survival, and reduce viral shedding in dogs with CDV.

Kanin Distemperde Ribavirin ve Proantosiyanidin'in Klinik Bulgular, Hematolojik ve Biyokimyasal Parametreler ve Viral Saçılım Üzerine Etkilerinin Değerlendirilmesi

Anahtar Kelimeler
Antiviral ajanlar, Canine distemper virus, Proantosiyanidinler, Ribavirin

Öz: Bu çalışma, canine distemper virus (CDV) ile doğal olarak enfekte olan köpeklerde ribavirin, proantosiyanidin ve ribavirin-proantosiyanidin kombinasyonunun etkilerini araştırdı. Her biri 2-6 aylık yaşta altı köpekten oluşan bir sağlıklı kontrol ve dört hasta grubu olmak üzere beş grup oluşturuldu. On gün boyunca A grubuna klasik tedavi (KT) [sıvı tedavisi ve antibiyotik], A+R grubuna KT + ribavirin, A+P grubuna KT + proantosiyanidin, A+R+P grubuna KT + ribavirin-proantosiyanidin uygulandı. T0, T3, T7 ve T10 günlerinde hematolojik ve biyokimyasal analizler ve klinik skorlama yapıldı. Bu günlerde ve tedaviden sonraki 5. ve 10. günlerde CDV varlığına bakıldı. Klinik skorlama A+P, A+R, A ve A+R+P gruplarının sırasıyla en iyi klinik iyileşmeyi gösterdiğini ortaya koydu. A+R grubunda toplam lökosit, nötrofil ve monositlerde düşüş gözlendi ($P<0.05$). Kreatin kinaz ve kreatin kinaz-myokardiyal band aktivitesi sadece A grubunda anlamlı olarak yüksek bulunurken ($P<0.01$), bu değerler sadece A+R grubunda tedavi ilerledikçe azaldı. Kreatinin değerleri A+P ve A+R gruplarında yüksekti ($P<0.01$). En düşük kalsiyum değeri A+R grubundaydı ($P<0.01$). Viral saçılım açısından en az CDV varlığı A+R grubunda tespit edildi. Sonuç olarak, CDV'li köpeklerde ribavirin veya proantosiyanidin kullanımı, klinik bulguların ciddiyetini azaltmaya, hayatta kalma oranını artırmaya ve viral saçılımı azaltmaya yardımcı oldu.

1. INTRODUCTION

Canine distemper (CD) is a worldwide, multisystemic, and potentially fatal viral disease of dogs. The causative agent is the *canine distemper virus* (*CDV*) which belongs to the genus morbillivirus from the *Paramyxoviridae* family [1,2]. CDV is an enveloped virus with a single-stranded, linear, negative-sense RNA genome [3].

Canine distemper is mostly transmitted via oronasal aerosols [4]. Viral shedding starts up to 5 days after the infection forms -before the clinical signs appear- with all excreta and secretions [5,6]. The duration of viral shedding might range from 1-2 weeks to 4 months [5,7]. Dogs of all ages are susceptible to the disease, but dogs aged 3-6 months are more vulnerable [7,8]. While most dogs develop a subclinical infection, only a few develop a rapidly progressive infection followed by death [5]. The clinical manifestations of CD are mainly related to the respiratory, gastrointestinal, and central nervous systems. During the acute infection, various clinical findings such as cutaneous rash, oculonasal discharge, conjunctivitis, anorexia, secondary bacterial infections, and neurological disorders occur [9]. Typical hematological findings are anemia, thrombocytopenia, absolute lymphopenia, neutropenia, and monocytopenia, especially in dogs with acute CDV infection [10]. Early cases are characterized by lymphopenia while late cases are by lymphocytosis [11]. Serum biochemical changes are usually nonspecific [12]. Hypoalbuminemia, hyperglobulinemia [13], or hypocalcaemia [13,14] have been reported. Some dogs may experience slight elevations in liver enzyme activity due to hypoxia or secondary infections caused by intestinal bacteria translocation [5].

Most treatments are ineffective; however, they should contain broad-spectrum antibiotics, balanced electrolyte solutions, and corticosteroids in some neurological form [4]. The clinical use of antiviral drugs in veterinary medicine is not common, and the number of controlled studies on the efficacy of these drugs is limited [15]. Ribavirin is an antiviral with a broad spectrum of activity against many RNA and DNA viruses, both in-vitro and in-vivo. The only commercially available and used compound with known antiviral activity against various members of the *Paramyxoviridae* family is ribavirin [16–18]. Ribavirin has been used in-vitro to treat CD and is reported to be very effective in inhibiting CDV replication [19]. Proanthocyanidin, a dimeric procyanidin obtained from the condensation of monomeric flavanols [20,21] possesses antioxidant, antibacterial, and antiviral activities [22–25] as well as immunomodulatory properties [26]. Proanthocyanidin has been found to inhibit CDV in vitro both at the early and late stages of viral replication [27].

To our knowledge, ribavirin and proanthocyanidin have not been used in clinical studies to treat dogs naturally infected with CDV. Therefore, this study aimed to evaluate how the therapeutic use of these medications affects clinical outcomes, hematological and biochemical parameters, and viral shedding. It was hypothesized that ribavirin would reduce viral shedding and increase survival rates. Likewise, it was assumed that proanthocyanidin would show similar effects thanks to its multiple properties.

2. MATERIAL AND METHOD

This study was approved by the Atatürk University Animal Experiments Local Ethics Committee (Decision number 2017/62), and for each dog, written informed consent was obtained from the owner.

2.1. Animals and Protocol Design

The study included 30 dogs, 2-6 months old, of any breed and sex (Table 1). Based on clinical examination, rapid test kit (Anigen Rapid CDV Ag Test Kit, Bionote, Korea) and PCR analysis results, complete blood cell count, and treatment applied, the dogs were divided into five groups A (n=6), A+R (n=6), A+P (n=6), A+R+P (n=6) and healthy (n=6). Dogs showing neurological signs before and at the beginning of treatment and previously vaccinated against CDV were excluded from the groups. All dogs received single subcutaneous ivermectin (0.2 mg/kg; Alfamec® 1%, Ege Vet) and a tablet comprising fenbendazole, pyrantel pamoate, and praziquantel (1 tablet/10 kg; Caniverm® 700 mg, Intermed) was given orally. The day after this procedure was regarded as the pre-treatment (T0). Dogs in the A group received the classical treatment [intravenous fluid therapy and antibiotic (7 mg/kg amoxicillin + 1.75 mg/kg clavulanic acid, once a day, subcutaneously) [28], (Synulox®, Zoetis, USA)] for ten days. In addition to the classical treatment, dogs in the A+R group received 30 mg/kg [29] ribavirin tablet (Copegus®, Roche, ABD), and dogs in the A+P group received 10 mg/kg [30] proanthocyanidin film tablet (Proanthocyanidin®, GNC, ABD), and dogs in the A+R+P group received 30 mg/kg ribavirin and 10 mg/kg proanthocyanidin for ten days. A detailed clinical examination was performed on each dog before and during treatment. The scoring system developed by Gill et al. [31] was modified, and a scoring table was created by grading rectal temperature, general condition, appetite, dehydration, vomiting, diarrhea, cough, ocular discharge, nasal discharge, dental problems, skin problems, neurological findings and death (Table 2).

2.2. Blood Sampling

Blood samples were taken from CDV-positive dogs on days 0 (T0), 3 (T3), 7 (T7), and 10 (T10) of treatment, whereas healthy dogs only had blood samples taken once. The *vena cephalica antebrachia* of all dogs was punctured, and blood samples were collected into EDTA vacutainers (Vacutainer, K2E 3.6 mg, BD, UK) and plain tubes (Vacutainer, BD, UK) for hematological and biochemical analyses. After leaving for ten minutes at room temperature for clotting, sera were obtained by centrifugation (Beckman Coulter, Allegra® X-30R, USA) at 3000 rpm for 10 minutes and stored at -80°C until being analysed. Hematological analyses were performed immediately.

2.3. Hematological Analyses

White blood cell (WBC), lymphocyte (LYM), monocyte (MON), neutrophil (NEU), eosinophil (EOS), basophil (BAS), red blood cell (RBC), and hemoglobin (HGB) counts, haematocrit (HCT), and platelet (PLT) levels were determined by a hematology analyser (Abacus Junior Vet5, Hungary).

Table 1. Breed, age and sex of dogs in groups.

Animal No	Control	A	A+R	A+P	A+R+P
1	Mix, 2 mo, m	Kangal Mix, 2 mo, f	BC, 3 mo, male	Kangal, 2 mo, f	Mix, 3 mo, m
2	Mix, 5 mo, f	Mix, 4 mo, f	Terrier Mix, 6 mo, f	Mix, 3 mo, f	Mix, 4 mo, f
3	Mix, 2 mo, f	Kangal Mix, 2 mo, m	Kangal Mix, 4 mo, f	Mix, 2 mo, f	Mix, 5 mo, f
4	Mix, 4 mo, m	Mix, 2.5 mo, m	Mix, 2 mo, m	Mix, 4 mo, m	Mix, 2 mo, f
5	Mix, 2 mo, f	Mix, 2.5 mo, f	Mix, 4 mo, m	Mix, 5 mo, m	Mix, 3 mo, m
6	Kangal, 4 mo, f	Mix, 5 mo, f	Kangal Mix, 4.5 mo, f	Mix, 6 mo, m	Russian Poodle, 5.5 mo, m

m: male; f: female; mo: month; BC: Border Collie

Table 2. Scoring system for clinical signs of canine distemper.

Signs	Score Value	Signs	Score Value	Signs	Score Value	Signs	Score Value	Signs	Score Value	Signs	Score Value	Signs	Score Value
Rectal temperature		**Nasal discharge**		**Ocular discharge**		**General condition**		**Appetite**		**Dehydration**		**Skin problems**	
37.1-39.3°C	0	No	0	No	0	Very good	0	Very good	0	No	0	No	0
39.4-39.9°C	1	Serous	1	Mild	1	Good	1	Good	1	Mild	1	Paw pad hyp	3
40.0-40.5°C	2	Seromucous	1	Moderate	2	Bad	2	Bad	2	Moderate	2	Nasal hyp	3
≥40.5°C	3	Mucous	2	Severe	3	Very bad	3	Very bad	3	Severe	3	ST-pustule	1
≤37.0°C	3	Mucopurulent	3										

Signs	Score Value	Signs	Score Value	Signs	Score Value	Signs	Score Value	Signs	Score Value	Signs	Score Value
Cough		**Fecal consistency**		**Vomiting**		**Neurologic findings**		**Dental problems**		**Death**	
No	0	Normal	0	No	0	No	0	No	0	No	0
Present	2	Watery	1	Present	1	Present	3	E and D hypoplasia	3	Present	20
Provoked cough	1	Bloody	3								

ST: skin thickening; E: enamel; D: dentin; Hyp: hyperkeratosis

2.4. Biochemical Analyses

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), creatinine (CREA), blood urea nitrogen (BUN), creatinine kinase (CK), creatinine kinase-MB (CK-MB), total protein (TP), albumin (ALB), amylase (AMY), cholesterol (CHOL), triglyceride (TRIG), glucose, phosphorus (P), calcium (Ca) and magnesium (Mg) concentrations in serum samples were determined by commercial kits using a biochemistry autoanalyzer (Mindray BS-300 Chemistry Analyser, China).

2.5. Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) Assay

2.5.1. Preparation of samples

Leukocyte samples and mucosal swab samples (combination of conjunctiva and nose) from dogs were used to test for CDV on days T0, T7, and T10, as well as on days 5 (PT5) and 10 (PT10) post-treatment. Mucosal swab samples were vortexed in 1 mL of phosphate-buffered saline (PBS) and centrifuged at 3000 rpm for 5 min. After centrifugation, 200 µl of supernatant was kept at -20 °C until extraction. Blood samples collected in EDTA vacutainers were centrifuged at 2000 rpm for 10 minutes. The buffy coat layer was placed in a tube containing 200 µl of isotonic PBS. Collected leukocytes were kept at -20º C until extraction.

2.5.2. Extraction

The samples were removed from -20 ºC and dissolved at room temperature. After dissolution, 200 µl of each sample was taken into a new tube, and the extraction process was carried out using the GF-1 Viral Nucleic Acid Extraction kit (Cat: GF-RD-100, Vivantis, Malaysia) according to the manufacturer's protocol. The samples were stored at -20°C until cDNA synthesis after extraction.

2.5.3. RT reaction (Complementary DNA synthesis, cDNA)

A reverse transcriptase enzyme was used to convert possible RNA to DNA after extraction. This process was carried out with the RevertAid First Strand cDNA Synthesis Kit (Cat. No. K1621, Thermo Fisher Scientific, Germany) according to the method prescribed by the company. The resulting cDNAs were stored at -20 ºC until the PCR process.

2.5.4. PCR analyses

PCR reaction was conducted to obtain the relevant product of the hemagglutinin gene region of *Paramyxovirus*, using the primer and method reported by Trebbien et al. [32]. Positive PCR amplicons were separated on a 1.5% agarose gel by gel electrophoresis, and displayed under UV light (Figure 1).

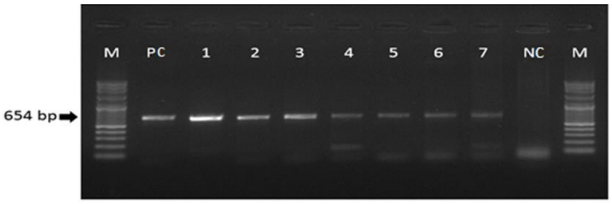

Figure 1. Gel image of PCR products made with the primers Zhao2010fwd and Bolt1997rev in the study of Trebbien et al. [32] with a product size of 654 bp. M: Marker (100 bp DNA Ladder), PC: Positive Control, NC: Negative Control, Study examples 1-3 (strong positive samples) and 4-7 (Weak positive samples).

2.6. Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics 25.0 (SPSS Inc., Chicago, IL, USA). The Shapiro-Wilk test was used to assess the distribution of data between groups, and it was determined that the data had a normal distribution. As a result, the One-way ANOVA was used to assess the data statistically. The statistical analysis evaluated treatments (A, A+R, A+P, A+R+P, and control groups) and sampling days (T0, T3, T7, and PT10) as primary effects, and the main effects were analysed independently. First, comparisons were done across treatment groups and then between data from the T0, T3, T7, and T10 sampling days within the same group. When the F-test for the main effect was significant, Duncan's Multiple Comparison Test was used to compare the means of the subclasses. All results were presented as the mean ± standard deviation (SD). A significance level of $P< 0.05$ was used for all statistical comparisons.

3. RESULTS

3.1. Clinical Status of the Dogs

A runny nose was observed in 19 of 24 dogs with CDV at T0. Ocular discharge and eye crust (16/24), high fever (14/24), cough, diarrhea, dehydration (12/24), skin problems (9/24), death (9/24), poor overall condition (4/24), vomiting, lack of appetite (2/24) and dental problems (2/24) were other clinical findings in dogs. According to clinical scores, the groups were the A+R+P group (349), A group (244), A+R group (188), and A+P group (182), in that order (Table 3). The difference between T10 and T0 was most pronounced in the A+R+P group, followed by the A group. The increase in the A+R and A+P groups was close to each other.

Table 3. Clinical score values of the treatment days of the groups.

Groups	T0	T3	T7	T10	Total
A	41	37	72	94	**244**
A+R	51	27	48	62	**188**
A+P	45	37	44	56	**182**
A+R+P	63	68	100	118	**349**

Following the initiation of the therapy, the general health of the dogs in the A+R+P group gradually declined. They also experienced a loss of appetite, bloody feces, severe dehydration, and mucopurulent nasal discharge. Dogs in group A had no improvement in cough despite treatment, nasal discharge, which was mostly serous at first, later turned into a mucopurulent consistency, and their general condition mostly deteriorated. In most dogs in the A+R group, prominent clinical findings at T0 were diarrhea and goopy eye discharge, and these problems mostly disappeared with treatment. Nasal discharge was a common clinical finding at T0 in all dogs in the A+P group. Even though the runny nose persisted throughout the treatment, the dogs' overall health improved significantly.

At T10, all dogs' body weights were generally reduced compared to T0 (Figure 2). In turn, the A+R and A+R+P groups had the most significant weight loss. The average body weight reduction was lowest in the A+P and A groups, respectively, and these groups often remained at their T0 weight.

During the 10-day treatment period, six out of six dogs (100%) in the A+P group, four out of six (46.6%) in the A and A+R groups, and two out of six (33.3%) in the A+R+P group survived.

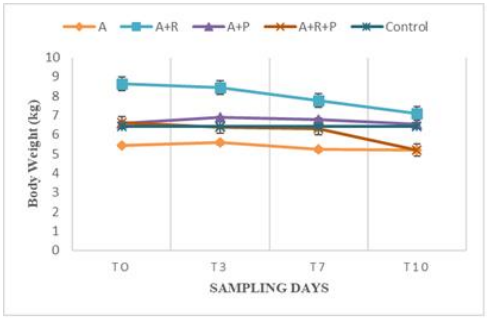

Figure 2. Mean body weight changes of the groups

3.2. Hematological Findings

In the A+R and A+R+P groups, WBC, LYM, NEU, and MON levels dropped as treatment progressed. However, it was found that in the A and A+P groups, these values increased as the treatment progressed. As the treatment progressed, a statistically significant decrease was detected in the levels of WBC, NEU ($P<0.05$) and MON ($P<0.01$) only in the A+R group. All patient groups had lower LYM counts than the control group on all treatment days.

All patient groups' RBC, HGB, and HCT values decreased statistically insignificantly during the treatment. The erythrocyte parameters of the A+R and A+R+P groups were generally higher than those in the A, A+P, and control groups. While PLT values increased gradually for group A, they decreased in other patient groups. Only the PLT values of the A group at T10 were the highest of all the groups ($P<0.05$). The statistically insignificant lowest PLT value in T10 was found in the A+R group (Table 4).

3.3. Biochemical Findings

During treatment, groups A and A+R+P had the highest ALT values ($P<0.05$), but ALT values decreased as treatment progressed. The A+R and A+P groups had very similar ALT values to the control group on all treatment days. Only in group A, the ALP value increased as the treatment days progressed ($P<0.05$). The group with the lowest ALP values was determined as the A+R group ($P<0.05$). The AST values of the A and A+R+P groups were higher than the control group and increased as the treatment progressed. AST values of the A+R group decreased. CK and CK-MB values of all patient groups were higher than the control group. Only in the A+R group did CK and CK-MB values decline with time and get close to the control group levels. The highest CK and CK-MB values were found in group A ($P<0.01$). The CREA levels of the A and A+R+P groups

were similar to those of the control group on all treatment days, but the CREA values were the highest in the A+P and A+R groups, respectively (P<0.01). As treatment progressed, CREA values increased only in the A+R group. CHOL values showed insignificant increases in all groups, but the groups with the least increase were A+R+P and A+P groups, respectively. Glucose values of all groups were found to be lower than the control group on all treatment days. While AMY values increased gradually in the A group, they first increased and then decreased in the other groups.

Table 4. Clinical score values of the treatment days of the groups.

		Groups					
Parameters	Day	A	A+R	A+P	A+R+P	Control	P
WBC	0	10.56±3.31	13.24±5.10^{A}	13.82±13.81	24.94±20.49	11.52±3.07	>0.05
(x10^3 µL)	3	12.89±8.53	8.26±2.83^{B}	11.67±5.99	18.33±7.96	11.52±3.07	>0.05
	7	18.19±11.37	5.89±2.64^{B}	16.06±8.49	12.62±7.43	11.52±3.07	>0.05
	10	17.91±9.91	7.63±2.41^{B}	17.87±9.45	12.90±17.44*	11.52±3.07	>0.05
	P	>0.05	**<0.05**	>0.05	>0.05	>0.05	
LYM	0	1.14±0.39	0.99±0.68	0.91±0.35	2.74±4.60	3.18±1.91	>0.05
(x10^3 µL)	3	1.51±0.62	1.09±0.83	0.98±0.37	2.22±2.28	3.18±1.91	>0.05
	7	2.54±2.82^{ab}	0.80±0.55^{b}	0.85±0.35^{b}	0.76±0.45^{b}	3.18±1.91^{a}	**<0.05**
	10	2.89±2.60	1.10±0.75	1.57±1.10	0.045±0.049*	3.18±1.91	>0.05
	P	>0.05	>0.05	>0.05	>0.05	>0.05	
NEU	0	8.65±3.12	11.16±4.69^{A}	11.93±12.83	19.28±14.72	7.53±1.60	>0.05
(x10^3 µL)	3	10.46±7.57	6.42±2.63^{B}	9.92±5.64	14.22±7.82	7.53±1.60	>0.05
	7	14.70±9.19^{a}	4.47±2.59^{B}	14.04±8.23^{a}	10.62±6.41^{ab}	7.53±1.60^{ab}	**<0.05**
	10	13.93±7.48^{a}	5.52±1.52^{B}	15.20±8.22^{a}	11.89±16.24*	7.53±1.60^{ab}	**<0.05**
	P	>0.05	**<0.05**	>0.05	>0.05	>0.05	
RBC	0	4.69±0.58^{b}	5.18±0.76^{b}	5.46±0.86^{b}	6.44±0.78^{a}	4.88±0.40^{b}	**<0.01**
(x10^6 µL)	3	4.61±0.47^{b}	5.34±0.86^{ab}	5.07±0.75^{b}	5.98±0.30^{a}	4.88±0.40^{b}	**<0.01**
	7	4.55±0.56	5.11±0.96	4.81±1.00	5.78±0.58	4.88±0.40	>0.05
	10	4.26±0.55	5.00±1.62	4.30±0.77	5.41±0.48*	4.88±0.40	>0.05
	P	>0.05	>0.05	>0.05	>0.05	>0.05	
HGB	0	8.38±1.38^{b}	10.03±1.60^{b}	9.78±1.68^{b}	12.56±2.39^{a}	9.21±1.17^{b}	**<0.01**
(g/dL)	3	7.88±0.98^{c}	10.31±2.13^{ab}	9.15±1.74^{bc}	11.73±1.42^{a}	9.21±1.17^{bc}	**<0.01**
	7	7.66±1.10^{b}	9.65±2.31^{ab}	8.71±1.93^{b}	11.05±1.07^{a}	9.21±1.17^{ab}	**<0.05**
	10	7.38±1.30	9.02±3.14	7.50±1.32	10.70±0.70*	9.21±1.17	>0.05
	P	>0.05	>0.05	>0.05	>0.05	>0.05	
HCT	0	28.86±4.88^{b}	35.57±6.07^{ab}	34.72±6.49^{ab}	41.09±6.52^{a}	33.29±5.19^{b}	**<0.05**
(%)	3	27.56±4.08^{b}	35.89±6.18^{a}	31.65±6.16^{ab}	38.12±3.97^{a}	33.29±5.19^{ab}	**<0.05**
	7	26.79±4.73	34.39±7.55	29.71±7.38	36.51±3.81	33.29±5.19	>0.05
	10	24.89±4.24	33.11±12.45	26.62±5.77	34.96±4.04*	33.29±5.19	>0.05
	P	>0.05	>0.05	>0.05	>0.05	>0.05	
PLT	0	424±268	389±53	456±190	401±132	376±151	>0.05
(x10^3 µL)	3	444±261	444±118	317±209	415±244	376±151	>0.05
	7	484±143	343±164	252±172	268±205	376±151	>0.05
	10	675±234^{a}	294±219^{b}	379±212^{b}	321±181*	376±151^{b}	**<0.05**
	P	>0.05	>0.05	>0.05	>0.05	>0.05	

WBC: white blood cell; LYM: lymphocyte; NEU: neutrophil, RBC: red blood cell; HGB: haemoglobin; HCT: haematocrit; PLT: platelet. $^{A, B}$ The means shown in different capital letters within the group (in the column) are statistically significant. $^{a, b}$ The means shown in different lowercase letters between the groups (on the line) are statistically significant. *The group A+R+P data from the 10th day were not statistically analysed because 2 dogs were still alive as a result of death in group A+R+P. Data are presented as the mean ± standard deviation

The highest AMY values were in the A+R+P group and this elevation was significant compared to the A and control groups (P<0.05). ALB values were low in all patient groups, but the low ALB values of the A+R and A+P groups at T0 and T10 were significant (P<0.05). P, Ca, and Mg values decreased numerically in the patient groups, but the A+R group's Ca value was lower on all treatment days compared to the control and A groups (P<0.01) (Table 5).

3.4. Virological Findings

Table 6 displays the PCR results for samples of mucosal swabs and leukocytes collected at T0, T7, T10, PT5, and PT10. In PCR analysis performed at T0, CDV was detected in mucosal swap samples but not in leukocyte samples in 6 of 24 dogs with CDV. On the other hand, CDV was found at T7 in the leukocyte samples of these six dogs. In four dogs in group A and two in the other groups, the viral load increased in leukocytes and mucosal swap samples in T7 and T10 compared to T0. When the PCR results of the groups were examined, the presence of CDV initially disappeared in the mucosal swap samples and then in the leukocyte samples. Among the treatment groups, the A+R and A+P groups were the best at reducing or stopping viral shedding, respectively. The CDV rapid test kit results were 100% accurate compared to the PCR results.

4. DISCUSSION

The results of this study revealed that ribavirin or proanthocyanidin may have positive effects on clinical outcomes, survival rate, viral shedding, and hematological and biochemical parameters in dogs naturally infected with CDV, consistent with the hypothesis.

In this study, 24 CDV-infected dogs exhibited various clinical symptoms in line with previous studies [33,34]. Ertürk [35] found that using antivirals like interferon,

Table 5. Comparison of biochemical parameters of the groups.

		Groups					
Parameters	Day	A	A+R	A+P	A+R+P	Control	P
ALT	0	39.00±30.17^{a}	15.50±8.36^{b}	16.17±7.67^{b}	31.33±6.18^{ab}	15.83±8.97^{b}	**<0.05**
(U/L)	3	27.83±12.27^{a}	14.67±14.25^{b}	14.00±6.95^{b}	29.50±4.27^{a}	15.83±8.97^{b}	**<0.05**
	7	26.33±9.97^{ab}	16.00±8.92^{b}	17.17±7.88^{b}	28.50±6.31^{a}	15.83±8.97^{b}	**<0.05**
	10	28.60±8.98^{a}	17.75±5.96^{b}	15.50±4.50^{b}	34.00±8.48*	15.83±8.97^{b}	**<0.05**
	P	>0.05	>0.05	>0.05	>0.05	>0.05	
ALP	0	153±27^{Ba}	88±52^{b}	160±53^{a}	131±50^{ab}	158±33^{a}	**<0.05**
(U/L)	3	147±26^{B}	106±56	174±32	158±71	158±33	>0.05
	7	171±23^{AB}	109±66	155±41	148±62	158±33	>0.05
	10	192±27^{Aa}	77±83^{b}	149±40^{a}	127±141*	158±33^{a}	**<0.05**
	P	**<0.05**	>0.05	>0.05	>0.05	>0.05	
CK	0	332±117	311±166	343±237	402±274	183±15	>0.05
(U/L)	3	466±184^{a}	341±122^{ab}	314±74^{bc}	212±60^{bc}	183±15^{c}	**<0.01**
	7	443±187	239±73	270±104	524±565	183±15	>0.05
	10	461±177^{a}	183±56^{b}	435±223^{a}	730±371*	183±15^{b}	**<0.01**
	P	>0.05	>0.05	>0.05	>0.05	>0.05	
CK-MB	0	81±31	129±118	75±54	88±69	54±13	>0.05
(U/L)	3	110±50^{a}	80±34^{ab}	62±23^{b}	45±11^{b}	54±13^{b}	**<0.01**
	7	106±48	57±15	57±30	104±116	54±13	>0.05
	10	113±46	53±25	94±55	137±50*	54±13	>0.05
	P	>0.05	>0.05	>0.05	>0.05	>0.05	
CREA	0	0.67±0.17^{b}	1.44±1.49^{b}	2.71±1.11^{a}	0.63±0.14^{b}	0.62±0.15^{b}	**<0.01**
(mg/dl)	3	0.61±0.07^{c}	1.96±1.37^{b}	3.40±0.84^{a}	0.69±0.16^{c}	0.62±0.15^{c}	**<0.01**
	7	0.64±0.15^{c}	1.67±1.56^{b}	3.02±0.60^{a}	0.44±0.22^{c}	0.62±0.15^{c}	**<0.01**
	10	0.55±0.14^{b}	2.27±2.01^{a}	2.55±0.96^{a}	0.58±0.17*	0.62±0.15^{b}	**<0.01**
	P	>0.05	>0.05	>0.05	>0.05	>0.05	
CHOL	0	309±82^{a}	124±35^{b}	182±52^{b}	336±29^{a}	319±43^{a}	**<0.01**
(mg/dl)	3	318±77^{a}	181±49^{b}	188±31^{b}	346±64^{a}	319±43^{a}	**<0.01**
	7	388±84^{a}	175±41^{b}	206±69^{b}	330±61^{a}	319±43^{a}	**<0.01**
	10	379±68^{a}	206±66^{b}	197±55^{b}	351±25*	319±43^{a}	**<0.01**
	P	>0.05	>0.05	>0.05	>0.05	>0.05	
GLUCOSE	0	80.82±28.12^{ab}	62.83±19.04^{b}	69.55±13.60^{b}	78.76±13.25^{ab}	94.30±6.42^{a}	**<0.05**
(mg/dl)	3	71.60±13.55^{b}	81.46±13.55^{ab}	76.26±3.45^{b}	76.67±17.59^{b}	94.30±6.42^{a}	**<0.05**
	7	80.82±25.51^{abc}	69.55±6.35^{bc}	65.21±12.09^{c}	95.40±33.07^{a}	94.30±6.42^{ab}	**<0.05**
	10	55.47±15.39^{c}	79.30±11.38^{ab}	64.13±18.65^{bc}	58.17±18.98*	94.30±6.42^{a}	**<0.01**
	P	>0.05	>0.05	>0.05	>0.05	>0.05	
AMY	0	365±132	357±367	493±300	668±391	330±65	>0.05
(U/L)	3	459±271^{b}	458±352^{b}	610±346^{ab}	923±356^{a}	330±65^{b}	**<0.05**
	7	405±98^{b}	519±348^{ab}	337±104^{b}	759±347^{a}	330±65^{b}	**<0.05**
	10	466±182	359±107	350±146	718±494*	330±65	>0.05
	P	>0.05	>0.05	>0.05	>0.05	>0.05	
ALB	0	2.35±0.18^{ab}	1.90±0.619^{c}	2.05±0.18^{bc}	2.38±0.09^{Aab}	2.46±0.15^{a}	**<0.05**
(mg/dl)	3	2.40±0.23^{a}	2.41±0.22^{a}	1.86±0.20^{b}	2.38±0.19^{Aa}	2.46±0.15^{a}	**<0.01**
	7	2.33±0.28	2.13±0.48	1.95±0.40	2.11±0.22^{B}	2.46±0.15	>0.05
	10	2.30±0.27^{ab}	2.07±0.33^{bc}	1.85±0.33^{c}	2.20±0.28*	2.46±0.15^{a}	**<0.01**
	P	>0.05	>0.05	>0.05	**<0.05**	>0.05	
Ca	0	14.31±5.26^{a}	6.80±1.94^{c}	4.93±0.86^{c}	10.23±1.98^{b}	11.21±1.20^{ab}	**<0.01**
(mg/dl)	3	10.91±1.91^{a}	6.61±1.65^{bc}	5.36±1.17^{c}	8.10±2.06^{b}	11.21±1.20^{ab}	**<0.01**
	7	12.85±2.88^{a}	6.90±2.64^{c}	5.03±1.07^{c}	9.48±1.30^{b}	11.21±1.20^{ab}	**<0.01**
	10	11.44±2.90^{a}	3.80±2.54^{b}	4.83±1.12^{b}	9.15±0.49*	11.21±1.20^{ab}	**<0.01**
	P	>0.05	>0.05	>0.05	>0.05	>0.05	

ALT: Alanine aminotransferase; ALP: alkaline phosphatase; CK: creatinine kinase; CK-MB: creatinine kinase-MB; CREA: creatinine; CHOL: cholesterol; AMY: amylase; ALB: albumin; Ca: calcium $^{A, B}$ The means shown in different capital letters within the group (in the column) are statistically significant. $^{a, b}$ The means shown in different lowercase letters between the groups (on the line) are statistically significant. *The group A+R+P data from the 10th day were not statistically analysed because 2 dogs were still alive as a result of death in group A+R+P. Data are presented as the mean ± standard deviation.

oseltamivir, and famciclovir results in the best clinical outcome for dogs with parvoviral enteritis. Similarly, the A+P and A+R groups achieved the best clinical scores in this study, respectively.

Several therapeutic benefits of proanthocyanidin [26,27], such as antibiotic, antiviral, antioxidant, and immunomodulatory, may have contributed to this situation. Ribavirin has an immunomodulatory effect [36] and improved the clinical course in infants with severe bronchiolitis caused by a respiratory syncytial virus [37]. The properties mentioned above of ribavirin and its being an effective antiviral against CDV [19] may have contributed to the second-best clinical score in the A+R group. However, using ribavirin was found to cause weight loss. Dogs in the A+R group lost the most weight, followed by those in the A+R+P group. Like this, Weiss et al. [38] found that cats administered ribavirin at doses of 11, 22, and 44 mg/kg progressively lost weight and their appetite. With therapy, the clinical score of the A+R+P group increased steadily, and the dogs' overall condition quickly deteriorated. The high clinical score of the A+R+P group at T0 (Table 3) and an adverse drug interaction may have led to this outcome. 10 mg/kg proanthocyanidin and 30 mg/kg ribavirin, which we used in this study, do not have such side effects.

Table 6. PCR analysis results of CDV presence in mucosal swab and leukocyte samples and visual evaluation of PCR positivity of the groups.

Viral Shedding		**1**		**2**		**3**		**4**		**5**		**6**	
Groups	**Day**	**MS**	**L**	**MS**	**L**	**MS**	**L**	**MS**	**L**	**MS**	**L**	**MS**	**L**
A	T0	++	+++++	++++	+++++	+	+	+++	N	+++++	N	+	+++
	T7	N	N	+++	+++++	+++++	+++++	+	++++	++	+++	++++	++++
	T10	D	D	+++++	+++	D	D	++++	+	N	N	+	+++
	PT5	D	D	D	D	D	D	++	++	N	N	++	+++
	PT10	D	D	D	D	D	D	+	+	D	D	+	+
A+R	T0	++++	+++++	+++++	++	+++++	++	+	+++	+	+++++	++++	++++
	T7	N	N	+++++	N	N	+	+	+++	N	+++++	+++++	+++++
	T10	N	N	D	D	D	D	+++	+++++	N	+++++	+++++	+++++
	PT5	N	N	D	D	D	D	++	+	N	++	++++	+++++
	PT10	N	N	D	D	D	D	D	D	N	N	D	D
A+P	T0	++++	+++++	+	N	++++	N	+	+++++	+++++	+++++	++++	+
	T7	++	+	++	++	++++	++++	+++++	+++++	+++++	+++++	+	+
	T10	N	N	+++++	+++++	+++++	+++++	+++++	+++++	++++	++++	+	+
	PT5	N	N	+++++	+++++	D	D	+	+++	+++++	+++++	+	+
	PT10	N	N	D	D	D	D	D	D	D	D	N	N
A+R+P	T0	+	+++++	+++	++++	++++	+++	++	++	++++	N	+++	N
	T7	+++++	+++++	N	N	+++++	++	++++	++++	++	++	N	++
	T10	D	D	N	N	D	D	D	D	D	D	N	N
	PT5	D	D	D	D	D	D	D	D	D	D	D	D
	PT10	D	D	D	D	D	D	D	D	D	D	D	D

MS: Mucosal Swabs, L: Leucocyte layer, N: Negative, D: Death

Proanthocyanidin has reportedly been used safely and effectively for 24 weeks in dogs at doses of 4, 20, or 40 mg/kg [30]. Ribavirin has been shown to cause anemia in dogs with CDV when taken at a dose of 30 mg/kg for 15 days [29]. There isn't a study that combines the usage of these two medications in dogs, though. To better understand drug interactions, studies involving larger numbers of animals in groups might be advantageous.

All patient groups had lymphopenia, which is consistent with CD findings [5,11,34,39]. When the effects of the treatments on the hematological parameters of the groups were examined, significant decreases in WBC, NEU ($P<0.05$), and MON ($P<0.01$) values were observed only in the A+R group, and this decrease was attributed to the effects of ribavirin. Because ribavirin use has been associated with leukopenia in both humans [40] and cats [39]. This view is supported by the gradual decline in all leukocyte parameters in the A+R+P group utilizing ribavirin. The findings demonstrated that 30 mg/kg of ribavirin caused leukopenia, neutropenia, and monocytopenia in CDV-positive dogs. The dogs in the A+P and A+R+P groups had higher leukocyte parameters than those in the A+R group. This elevation indicated that the leukopenia-inducing effects of ribavirin and CD might be mitigated by proanthocyanidin's immunomodulatory properties [41] and antioxidant properties [42], which protect leukocyte cells.

The gradual decrease in erythrocyte parameters in all patient groups during the treatment was consistent with the reports of anemia in CD [4,11,34]. According to reports, ribavirin is directly toxic to erythrocytes, induces dose-related hemolysis and has adverse effects such as hemolytic anemia and bone marrow suppression [43,44]. However, it was found in this study that the groups using ribavirin had higher erythrocyte parameters than the other groups. These findings suggest that 1) By inhibiting CDV replication, ribavirin lessens the degree of anemia associated with the disease. 2) Blood RBC, HGB, and HCT values in dogs are not affected by ten days of treatment with 30 mg/kg ribavirin. 3) Ribavirin does not accumulate much in canine erythrocytes. The last two inferences can be explained as follows: Different erythrocyte affinities for isolation ribavirin cause different degrees of hematological toxicity in different species [38,44]. Some species, such as mice and rats, accumulate less ribavirin in their erythrocytes

than humans, resulting in fewer hemolytic effects [38]. Ribavirin-induced anemia is most severe in monkeys, followed by humans, rodents, and dogs [45]. In conclusion, it can be concluded that ribavirin at a dose of 30 mg/kg is safe to use in dogs with CDV and lessens the severity of CD-related anemia without producing evident hemolytic anemia.

When the high leukocyte levels are considered, a secondary bacterial infection [6] may be the reason for the high PLT values in group A. The reduction in PLT values in ribavirin-treated groups during treatment is consistent with reports that ribavirin produces thrombocytopenia [38,40].

An elevated serum AST activity and a higher ALT than AST [46] and high GGT activity [47] indicate liver disease in dogs. As is well known, liver enzyme activity is slightly elevated in CDV infection [13]. The AST and GGT activities of the treatment groups in this study did not differ statistically significantly. However, the ALT values of the A and A+R+P groups were higher than those of the other groups ($P<0.05$). Thus, the virus's effects on the liver could generate an increase in ALT. It has been demonstrated that proanthocyanidin [30] and ribavirin [29] had no influence on these enzyme levels in dogs. Similarly, in this study, the ALT activity of the A+R and A+P groups was nearly equivalent to that of the control group but lower than that of the A group ($P<0.05$). Moreover, these groups' final GGT and ALP activities were lower than those of the control group, and their ALT activities were lower than their AST activities. These findings suggest that using ribavirin or proanthocyanidin may be beneficial in reducing disease-related ALT elevations and has no adverse effects on the liver.

ALP activity increased during treatment in group A. ($P<0.05$). Enteritis and osteoclast, osteoblast, and osteocyte degeneration and necrosis caused by CDV [48] were suspected as potential causes of this condition. ALP activity was lower in T10 in the A+R group than in the other groups ($P<0.05$). This decrease may be due to ribavirin use. Because ribavirin has been linked to reduced bone ALP isoenzyme activity in people with chronic hepatitis C infection [49,50].

CDV can cause myocarditis in young dogs, but the histological changes in the myocardium are mild compared to myocarditis caused by parvovirus [51]. Increases in CK levels have been reported in some dogs experimentally infected with CDV [52]. Compared with the control group, CK, CK-MB ($P<0.01$), and AST, markers of myocardial damage [53] were higher in group A, providing evidence of myocarditis in CDV infection. In patients with hantavirus renal syndrome disease, ribavirin therapy has reportedly been associated with a decrease in CK-MB activity [54]. Confirming this information, CK and CK-MB activities decreased numerically in the A+R group as treatment progressed. Thus, it can be claimed that the using ribavirin in CDV infection effectively prevents cardiac damage.

According to reports, the CREA values were unaffected by using ribavirin in dogs and cats [29,55] or proanthocyanidin in dogs [30]. However, since ribavirin is excreted through the kidneys, it tends to accumulate in the kidneys in the presence of renal dysfunction [56]. Only the CREA values of the A+R group increased gradually during treatment. Considering the decrease in AST, CK, and CK-MB levels in the A+R group as the treatment continued, it was thought that the high CREA values in T0 might have been caused by muscle damage, and the high on other days ($P<0.01$) might have resulted from kidney damage in which ribavirin also played a role.

Anorexia, protein-losing enteropathies, hepatic disorders, maldigestion, and severe malnutrition can all cause hypocholesterolemia in dogs [57]. The A+R and A+P groups' CHOL levels at T0 were lower than those of the control group ($P<0.01$), and diarrhea and maldigestion were assumed to cause this decline. The low glucose, ALB, and TP levels ($P<0.05$) of these groups at T0 also support this inference. Proanthocyanidin has been observed to markedly lower CHOL levels in rats [58,59]. The A group's CHOL levels increased during the treatment, but the A+R+P group's CHOL levels reduced, while the A+P group's increase was the smallest. Therefore, it may be asserted that the use of proanthocyanidin prevents the increase of CHOL levels in dogs with CDV.

Mild AMY activity elevation occurs in dogs with acute pancreatitis, acute enteritis, perforated duodenal ulcers, intestinal torsion, and infarctions [46]. In group A, AMY values gradually increased, and most dogs had mild diarrhea. Inclusion bodies have been found in pancreatic tissue associated with CD [60,61]. Therefore, it was assumed that CDV-affected pancreatic tissue and enteritis caused this rise. Studies show that proanthocyanidin effectively reduced high levels of AMY and alleviated pancreatic damage in rats with acute pancreatitis [62]. At a dose of 30 mg/kg administered to rats, ribavirin was likewise seen not to damage the pancreas [63]. The AMY values of the A+R and A+P groups at T10 approached the control group values. These findings concluded that treating ribavirin or proanthocyanidin prevented the disease's associated elevations in AMY. The A+R+P group had higher AMY activity at T3 and T7 than the A and control groups ($P<0.05$). At the same time, the most severe diarrhea cases were detected in this group. Thus, at high AMY levels, pharmacological interactions between ribavirin and proanthocyanidin, CDV's impact on pancreatic tissue, and the presence of severe enteritis may have played a role.

Albumin levels are mostly decreased in renal glomerular diseases, protein-losing enteropathies, malnutrition, and liver diseases [46]. Hypoalbuminemia has been reported to occur in CD [13]. The ALB values are reported to be unaffected by the usage of ribavirin [29] or proanthocyanidin [30]. ALB, TP, and glucose levels were lower ($P<0.05$), and CREA levels were higher ($P<0.01$) in the A+R and A+P groups compared to the

control group, while there was no significant change in liver enzyme activities. These findings led to the inference that low ALB levels in these group would be caused by infection, malnutrition, and renal injury. The gradual decrease in TP and ALB values in the A+R+P group was attributed to the dogs having severe enteritis. Dogs with CDV [13] and cats receiving ribavirin [55] had decreased blood Ca levels. The A+R group exhibited the lowest Ca values. Ribavirin and CDV infection were suspected of contributing to this decline, in addition to the low baseline value.

Reportedly, CDV can be detected in whole blood samples two days after experimental infection in dogs, peaking on day six and dramatically declining on day twelve [64]. The absence of CDV in the leukocyte layer of six dogs at T0 can be attributed to the dogs being generally asymptomatic and not in the viremia stage. The increase in viral load in more dogs in group A in the following days of treatment showed that classical treatment was insufficient to reduce viral shedding. The highly antiviral effect of ribavirin and proanthocyanidin against CDV has been demonstrated [19,27,65]. Supporting this information, the best groups in lowering or stopping viral shedding were A+R and A+P, respectively. As a result, it can be argued that using ribavirin or proanthocyanidin in CD reduces CDV shedding. In PT10, no dogs survived in the A+R+P group, but two dogs survived in the A, A+R, and A+P groups. While viral shedding continued in group A, it had ceased in the other groups. However, in the A+R group, viral shedding ended earlier (T7). This action suggested that ribavirin was more efficient than proanthocyanidin at lowering and preventing viral shedding.

The study's shortcomings include the small number of animals in each group and the lack of inflammatory markers to evaluate treatment efficacy. This study, however, is regarded significant in terms of analysing the impact of ribavirin and proanthocyanidin use on clinical outcomes, hematological and biochemical parameters, viral shedding, and side effects in CDV-infected dogs.

5. CONCLUSION

Classical treatment was unable to enhance clinical outcomes, lessen viral shedding, or prevent cardiac damage. In terms of survival rates, it produced the same outcomes as ribavirin, nevertheless. Ribavirin administration to CDV-positive dogs caused leukopenia and weight loss, decreased serum Ca levels and was associated with kidney damage. On the other hand, ribavirin had no adverse effect on erythrocyte parameters, reduced the severity of CD-related anemia, did not induce liver damage, and had a beneficial effect in preventing cardiac damage and the rise in disease-associated AMY activity. The administration of proanthocyanidin in CDV-positive dogs was found to have good effects on causing an increase in leukocyte counts, preventing the rise in disease-related AMY activity, reducing CHOL levels, and did not cause liver and kidney damage. Proanthocyanidin and ribavirin achieved the most remarkable improvements in clinical course and survival rates, respectively. Ribavirin and proanthocyanidin produced the best results in terms of reducing or stopping viral shedding, respectively. Therefore, to limit the spread of the disease, it was considered beneficial to include ribavirin in the treatment, particularly in areas where dogs are collectively sheltered. Ribavirin-proanthocyanidin therapy decreased viral shedding, mitigated the leukopenia-inducing effects of ribavirin, did not result in liver or kidney damage, but quickly worsened the dogs' general health. It is advised to carry out experiments with more animals in the groups to corroborate the findings of this study and to gain comprehensive information about the pharmacokinetics and toxicity of combining these two medications.

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REFERENCES

[1] Beineke A, Puff C, Seehusen F, Baumgartner W. Pathogenesis and immunopathology of systemic and nervous canine distemper. Vet Immunol Immunopathol. 2009;127(1–2):1–18.
[2] Carpenter MA, Appel MJ, Roelke-Parker ME, Munson L, Hofer H, East M, et al. Genetic characterization of canine distemper virus in Serengeti carnivores. Vet Immunol Immunopathol. 1998;65(2–4):259–66.
[3] Kingsbury DW. Paramyxoviridae. Intervirology. 1978;10(3):137–52.
[4] Greene CE, Vandevelde M. Canine distemper. In: Greene CE, editor. Infectious diseases of the dog and cat. 4 th. St Louis,: Saunders; 2012. p. 25–42.
[5] Sykes JE. Canine distemper virus infection. In: Sykes JE, editor. Canine and feline infectious diseases. 1 st. St Louis: Saunders; 2014. p. 152–65.
[6] Leisewitz AL, Carter A, van Vuuren M, van Blerk L. Canine distemper infections, with special reference to South Africa, with a review of the literature. J S Afr Vet Assoc. 2001;72(3):127–36.
[7] Martella V, Elia G, Buonavoglia C. Canine distemper virus. Vet Clin North Am Small Anim Pr. 2008;38(4):787–97, vii–viii.
[8] Taylor S. Encephalitis, myelitis and meningitis. In: Couto CG, Nelson RW, editors. Small Animal Internal Medicine. 4th ed. St. Louis, Missouri: Mosby Elsevier; 2009. p. 1059–62.
[9] Krakowka S, Axthelm MK, Johnson GC. Canine distemper virus. In: Olsen RG, Krakowka S, Blakeslee JR, editors. Comparative Pathobiology of Viral Diseases. Boca Raton: CRC Press; 1985. p. 137–64.

[10] Shell LG. Canine distemper. Compend Contin Educ Pract Vet. 1990;12(2):173–9.
[11] Ezeibe MCO, Udegbunam RI. Haematology of dogs infected with canine distemper virus. Sokoto J Vet Sci. 2008;7(2):32.
[12] Greene GE, Appel M. Canine Distemper Virus. In: Greene GE, editor. Infectious Disease of the Dog and Cat. 2nd ed. Philadelphia, PA: Saunders; 1998. p. 1–22.
[13] Appel MJ. Pathogenesis of canine distemper. Am J Vet Res. 1969;30(7):1167–82.
[14] Weisbrode SE, Krakowka S. Canine distemper virus-associated hypocalcemia. Am J Vet Res. 1979;40(1):147–9.
[15] Hartmann K. Antiviral and immunomodulatory chemotherapy. In: Greene CE, editor. Infectious diseases of the dog and cat. 2013. p. 10–24.
[16] De Clercq E, Cools M, Balzarini J, Snoeck R, Andrei G, Hosoya M, et al. Antiviral activities of 5-ethynyl-1-beta-D-ribofuranosylimidazole-4-carboxamide and related compounds. Antimicrob Agents Chemother. 1991;35(4):679–84.
[17] Shigeta S, Mori S, Baba M, Ito M, Honzumi K, Nakamura K, et al. Antiviral activities of ribavirin, 5-ethynyl-1-beta-D-ribofuranosylimidazole-4-carboxamide, and 6'-(R)-6'-C-methylneplanocin A against several ortho- and paramyxoviruses. Antimicrob Agents Chemother. 1992;36(2):435–9.
[18] del Toro-Riera M, Macaya-Ruiz A, Raspall-Chaure M, Tallada-Serra M, Pasqual-Lopez I, Roig-Quilis M. [Subacute sclerosing panencephalitis: combined treatment with interferon alpha and intraventricular ribavirin]. Rev Neurol. 2006;42(5):277–81.
[19] Elia G, Belloli C, Cirone F, Lucente MS, Caruso M, Martella V, et al. In vitro efficacy of ribavirin against canine distemper virus. Antivir Res. 2008;77(2):108–13.
[20] Husain SR, Cillard J, Cillard P. Hydroxyl Radical Scavenging Activity of Flavonoids. Phytochemistry. 1987;26(9):2489–91.
[21] Fine AM. Oligomeric proanthocyanidin complexes: history, structure, and phytopharmaceutical applications. Altern Med Rev. 2000;5(2):144–51.
[22] Cheng HY, Lin CC, Lin TC. Antiviral properties of prodelphinidin B-2 3'-O-gallate from green tea leaf. Antivir Chem Chemother. 2002;13(4):223–9.
[23] Iwasawa A, Niwano Y, Mokudai T, Kohno M. Antiviral Activity of Proanthocyanidin against Feline Calicivirus Used as a Surrogate for Noroviruses, and Coxsackievirus Used as a Representative Enteric Virus. Biocontrol Sci. 2009;14(3):107–11.
[24] Takeshita M, Ishida Y, Akamatsu E, Ohmori Y, Sudoh M, Uto H, et al. Proanthocyanidin from blueberry leaves suppresses expression of subgenomic hepatitis C virus RNA. J Biol Chem. 2009;284(32):21165–76.
[25] Xu XY, Xie HH, Wang YF, Wei XY. A-Type Proanthocyanidins from Lychee Seeds and Their Antioxidant and Antiviral Activities. J Agric Food Chem. 2010;58(22):11667–72.
[26] Zhang XY, Li WG, Wu YH, Zheng TZ, Li W, Qu SY, et al. Proanthocyanidin from grape seeds potentiates anti-tumor activity of doxorubicin via immunomodulatory mechanism. Int Immunopharmacol. 2005;5(7–8):1247–57.
[27] Gallina L, Dal Pozzo F, Galligioni V, Bombardelli E, Scagliarini A. Inhibition of viral RNA synthesis in canine distemper virus infection by proanthocyanidin A2. Antivir Res. 2011;92(3):447–52.
[28] Allerton F. BSAVA Small Animal Formulary Part A: Canine and Feline. 10th ed. Allerton F, editor. Gloucester: British Small Animal Veterinary Association; 2020. 98 p.
[29] Mangia SH, Moraes LF, Takahira RK, Motta RG, Franco MMJ, Megid J, et al. The side effects of ribavirin, prednisone and DMSO in dogs naturally infected by canine distemper virus. Pesqui Vet Bras. 2014;34(5):449–54.
[30] Martineau A, Leray V, Lepoudere A, Blanchard G, Bensalem J, Gaudout D, et al. A mixed grape and blueberry extract is safe for dogs to consume. BMC Vet Res. 2016;12(1):162.
[31] Gill M, Srinivas J, Morozov I, Smith J, Anderson C, Glover S, et al. Three-year duration of immunity for canine distemper, adenovirus, and parvovirus after vaccination with a multivalent canine vaccine. J Appl Res Vet Med. 2004;2(4):227–34.
[32] Trebbien R, Chriel M, Struve T, Hjulsager CK, Larsen G, Larsen LE. Wildlife reservoirs of canine distemper virus resulted in a major outbreak in Danish farmed mink (Neovison vison). PLoS One. 2014;9(1):e85598.
[33] Gemma T, Watari T, Akiyama K, Miyashita N, Shin YS, Iwatsuki K, et al. Epidemiological observations on recent outbreaks of canine distemper in Tokyo area. J Vet Med Sci. 1996;58(6):547–50.
[34] Salem NY. Canine viral diarrhea: clinical, hematologic and biochemical alterations with particular reference to in-clinic rapid diagnosis. Glob Vet. 2014;13(3):302–7.
[35] Ertürk N. Köpeklerde parvoviral enteritisin tedavisinde antiviral kullanımının etkinliğinin değerlendirilmesi. Sağlık Bilimleri Enstitüsü, Veteriner İç Hastalıkları Anabilim Dalı. [Tez]: Erzurum: Atatürk Üniversitesi; 2015.
[36] Ogbomo H, Michaelis M, Altenbrandt B, Doerr HW, Cinatl Jr. J. A novel immunomodulatory mechanism of ribavirin in suppressing natural killer cell function. Biochem Pharmacol. 2010;79(2):188–97.
[37] Lindgren C, Grogaard J. [Ribavirin treatment of children with respiratory syncytial virus bronchiolitis]. Tidsskr Nor Laegeforen. 1994;114(17):1928–9.
[38] Weiss RC, Cox NR, Boudreaux MK. Toxicologic effects of ribavirin in cats. J Vet Pharmacol Ther. 1993;16(3):301–16.
[39] Axthelm MK, Krakowka S. Canine distemper virus-induced thrombocytopenia. Am J Vet Res. 1987;48(8):1269–75.
[40] Aydın M, Aksöz E, Korkut O, Akhan S. Farklı Pegile İnterferon-α Molekülleriyle Ribavirin Kombinasyonlarının Hematolojik Yan Etkiler

Açısından Karşılaştırılması. Klimik Derg. 2014;27(3):99–102.
[41] Liu YZ, Cao YG, Ye JQ, Wang WG, Song KJ, Wang XL, et al. Immunomodulatory effects of proanthocyanidin A-1 derived in vitro from Rhododendron spiciferum. Fitoterapia. 2010;81(2):108–14.
[42] Huang Y, Zhao H, Cao K, Sun D, Yang Y, Liu C, et al. Radioprotective Effect of Grape Seed Proanthocyanidins In Vitro and In Vivo. Oxid Med Cell Longev. 2016;2016:5706751.
[43] Kowdley K V. Hematologic side effects of interferon and ribavirin therapy. J Clin Gastroenterol. 2005;39(1 Suppl):S3-8.
[44] Canonico PG, Kastello MD, Spears CT, Brown JR, Jackson EA, Jenkins DE. Effects of ribavirin on red blood cells. Toxicol Appl Pharmacol. 1984;74(2):155–62.
[45] Lin C-C, Yeh L-T, Luu T, Lourenco D, Lau JYN. Pharmacokinetics and metabolism of [(14)C]ribavirin in rats and cynomolgus monkeys. Antimicrob Agents Chemother. 2003;47(4):1395–8.
[46] Turgut K. Karaciğer Hastalıkları ve Testleri. In: Turgut K, editor. Veteriner Klinik Laboratuvar Teşhis. 2. Baskı. Konya: Bahçıvanlar Basım Sanayi A.Ş.; 2000. p. 202–57.
[47] Özkanlar Y. Laboratuvar Değerler ve Sonuçların Yorumlanması. In: Özkanlar Y, editor. Küçük Hayvan Medikal Ayırıcı Tanı Bir Liste Kitabı. 1. Baskı. Malatya: Medipres; 2016. p. 263–301.
[48] Baumgartner W, Boyce R, Alldinger S, Axthelm M, Weisbrode S, Krakowka S, et al. Metaphyseal bone lesions in young dogs with systemic canine distemper virus infection. Vet Microbiol. 1995;44(2–4):201–9.
[49] Abenavoli L, Mazza M, Almasio P. The optimal dose of ribavirin for chronic hepatitis C: From literature evidence to clinical practice: The optimal dose of ribavirin for chronic hepatitis C. Hepat Mon. 2011;11(4):240–6.
[50] Moreira RO, Balduíno A, Martins HSLH, Reis JSN, Duarte MEL, Farias MLF, et al. Ribavirin, but not interferon alpha-2b, is associated with impaired osteoblast proliferation and differentiation in vitro. Calcif Tissue Int. 2004;75(2):160–8.
[51] Ware W. Cardiovascular System Disorders. In: Nelson R, Couto C, editors. Small Animal Internal Medicine. 5th ed. Canada: Elsevier Mosby; 2014. p. 141.
[52] Higgins RJ, Krakowka S, Metzler AE, Koestner A. Canine distemper virus-associated cardiac necrosis in the dog. Vet Pathol. 1981;18(4):472–86.
[53] Bakirel U, Gunes S. Value of cardiac markers in dogs with chronic mitral valve disease. Acta Vet Brno. 2009;59(2–3):223–9.
[54] Zhang Q, Tang R, Yuan G. [The treatment effect of ribavirin to hemorrhagic fever with renal syndrome on the kinetics of serum creatine phosphate kinase isoenzyme]. Chin J Exp Clin Virol. 1999;13(3):266–8.
[55] Bogdanchikova N, Vázquez-Muñoz R, Huerta-Saquero A, Pena-Jasso A, Aguilar-Uzcanga G, Picos-Díaz P, et al. Silver nanoparticles composition for treatment of distemper in dogs. Intern J Nanotechnol. 2016;13:227–37.
[56] Jain AB, Eghtesad B, Venkataramanan R, Fontes PA, Kashyap R, Dvorchik I, et al. Ribavirin dose modification based on renal function is necessary to reduce hemolysis in liver transplant patients with hepatitis C virus infection. Liver Transpl. 2002;8(11):1007–13.
[57] Robbins R, Viviano KR. Hypocholesterolemia and nonregenerative, suspected immune-mediated, anemia: Report of 3 canine cases. Can Vet J. 2017;58(10):1100–4.
[58] Mansouri E, Khorsandi L, Zare Moaiedi M. Grape Seed Proanthocyanidin Extract Improved some of Biochemical Parameters and Antioxidant Disturbances of Red Blood Cells in Diabetic Rats. Iran J Pharm Res IJPR. 2015;14(1):329–34.
[59] El-Adawi H, Mohsen M, Youssef D, El-Sewedy S. Study on the Effect of Grape Seed Extract on Hypercholestrolemia: Prevention and Treatment. Int J Pharmacol. 2006;2(6):593–600.
[60] Woo G, Jho Y, Bak E. Canine Distemper Virus Infection in Fennec Fox (Vulpes zerda). J Vet Med Sci. 2010;72(8):1075–9.
[61] Caswell J, Williams K. Respiratory System. In: Maxie M, editor. Jubb, Kennedy, and Palmer's Pathology of Domestic Animals. 6th ed. China: Elsevier; 2016. p. 575.
[62] Akyuz C, Sehirli A, Topaloglu U, Ogunc A, Cetinel S, Sener G. Protective Effects of Proanthocyanidin on Cerulein-induced Acute Pancreatic Inflammation in Rats. Gastroenterol Res. 2009;2:20–8.
[63] Motor S, Alp H, Senol S, Pinar N, Motor V, Kaplan I, et al. Comparison of the chronic effects of ribavirin and caffeic acid phenethyl ester (CAPE) on pancreatic damage and hepatotoxicity. Int J Clin Exp Med. 2014;7:1005–13.
[64] Sehata G, Sato H, Ito T, Imaizumi Y, Noro T, Oishi E. Use of quantitative real-time RT-PCR to investigate the correlation between viremia and viral shedding of canine distemper virus, and infection outcomes in experimentally infected dogs. J Vet Med Sci. 2015;77(7):851–5.
[65] Carvalho O V, Saraiva GL, Ferreira CGT, Felix DM, Fietto JLR, Bressan GC, et al. In-vitro antiviral efficacy of ribavirin and interferon-alpha against canine distemper virus. Can J Vet Res. 2014;78(4):283–9.