



Changes in radiographic and electrocardiographic parameters in dogs with myxomatous mitral valve degeneration under the influence of humic substances

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Authors' Contributions:

RVV: Methodology; Investigation; Data curation; Formal analysis; Visualization; Writing — original draft, review and editing.

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The article presents the results of studying the effects of humic substances in dogs with stage C myxomatous mitral valve degeneration receiving standard therapy according to the recommendations of the American College of Veterinary Internal Medicine. Comprehensive assessment of radiographic and electrocardiographic parameters before and after treatment was performed. It was found that adding humic substances to the standard therapy contributed to reducing heart size, decreasing pulmonary congestion and improving electrophysiological characteristics of the heart. The obtained results indicate the advisability of using humic substances as an additional tool in the complex treatment of dogs with myxomatous mitral valve degeneration.

Key words: endocardiosis, physiology, antioxidants, cardiac arrhythmia, veterinary cardiology

Introduction

Myxomatous mitral valve degeneration (MMVD), also known as endocardiosis, is the most common form of heart valve pathology in dogs, causing heart failure and significantly deteriorating the animals' quality of life [16]. The disease is characterized by progressive degeneration of the connective tissue structures of the valve, leading to blood regurgitation, heart chamber dilation, and the development of pulmonary congestion. According to research data, MMVD is diagnosed in 70–80 % of dogs older than 10 years, especially small and medium breeds, making this pathology highly relevant for contemporary veterinary cardiology [4, 5].

According to recommendations by the American College of Veterinary Internal Medicine (ACVIM), standard therapy for dogs with chronic heart failure due to MMVD includes pimobendan, angiotensin-converting enzyme inhibitors (ACE inhibitors), diuretics (furosemide), and aldosterone antagonists (spironolactone). These medications effectively reduce congestion, improve hemodynamics, and enhance quality of life [8]. However, despite proven efficacy, their use is often associated with certain limitations, including potential side effects, electrolyte

imbalances, development of diuretic resistance, and inadequate management of oxidative stress, which plays a significant role in heart pathology progression.

Recently, more attention has been given to finding additional therapeutic agents capable of reducing the negative effects of oxidative stress, maintaining cardiomyocyte functionality, and slowing structural changes in the heart [2]. Humic substances, natural organic compounds characterized by high antioxidant activity and the ability to stabilize cell membranes, reducing myocardial and cardiac conduction system damage under chronic heart failure conditions, are of particular interest [19].

Previous studies, conducted in Ukraine and abroad, indicate the positive impact of humic substances on general antioxidant status, reduction of oxidative stress markers (malondialdehyde, MDA), and their ability to stabilize cardiac rhythm and reduce cardiac arrhythmias [1, 15]. However, comprehensive assessment of their effectiveness in dogs with MMVD, especially under standard therapy conditions, remains insufficiently studied.

The aim of our study was to determine the effects of humic substances on radiographic and electrocardiographic parameters in dogs with stage C MMVD undergoing standard therapy per ACVIM protocols.

Materials and Methods

The research was conducted from 2020 to 2022 involving dogs with MMVD at the veterinary clinical diagnostic center of Dnipro State Agrarian and Economic University and LLC “Veterinary Space Discovery” in Dnipro. Animals underwent clinical examinations, radiography, echocardiography, and electrocardiography. Animals were divided into three groups: standard therapy (ST, n=6), standard therapy with humic substances (STH, n=6), and control (n=6). Animals in the ST and STH groups received standard ACVIM-classified therapy, with the STH group additionally receiving “Humilid” orally at 10 mg/kg once daily before feeding. Exclusion criteria included recent specific treatments, oncology, infections, or severe illnesses.

Radiography of the thorax in the studied animals was performed in a single projection to reduce the cost of research during martial law. Animals were positioned in right lateral recumbency with maximum cranial extension of the forelimbs, using *Optima-xr220amx* (GE, USA) equipment and *Kodak DirectView CR975* automatic digitizing system (Carestream Health, USA). The analysis of radiographic images included assessment of the vertebral heart score (VHS) and vertebral left atrial size (VLAS). The radiological assessment of lung and vascular conditions was performed subjectively (semi-quantitatively) based on the severity of bronchial and interstitial patterns and by comparing the diameter of veins in the cranial lung lobes with artery diameters. All measurements were expressed in arbitrary units [3, 7].

To detect disturbances in conduction, excitation, and automaticity of the heart, the *Bioset 9000* apparatus (Hörmann, Germany) was used. Animals were positioned on the examination table in right lateral recumbency for ECG recording. Electrocardiograms were registered using standard Einthoven leads (I, II, III) and augmented Goldberger leads (aVR, aVL, aVF) on millimeter paper at a speed of 50 mm/s and a standard input voltage of 1 mV = 10 mm. ECG recordings lasted 5 minutes and were analyzed using an electrocardiographic ruler according to established algorithms [14]. During ECG analysis, the rhythm, R-R interval regularity, heart rate (beats/min), amplitude (mV), and duration (s) of the P wave, PQ interval, QRS complex, and ST segment were evaluated. Obtained data were compared with reference values from R. Santilli [14]. The functional state of the animals was assessed without using medications negatively affecting the vasomotor, respiratory centers of the brain, or the heart.

Statistical data processing was performed using *Microsoft® Excel®* (version 2412) in accordance with methodological recommendations. The number of animals was presented as absolute (n) and relative (%) values. Data were expressed as mean ± standard deviation (M±SD). The paired Student's *t*-test was used to evaluate dynamics of studied parameters in animal groups before and after therapy. Intergroup differences were analyzed using one-way ANOVA followed by Bonferroni retrospective tests.

Results and Discussion

At the initial stage (Day 1 of the study), animals in both the ST and STH groups exhibited pronounced changes in the form of increased bronchial and interstitial patterns (fig. 1). These changes indicated the presence of congestion and early pulmonary edema. Radiographically, the thickening of veins in the cranial lung lobes was approximately twice the diameter of the arteries (a vein to artery ratio of about 2:1), indicating venous congestion in the pulmonary circulation. By Day 21 of treatment, noticeable shifts toward normalization were observed, particularly

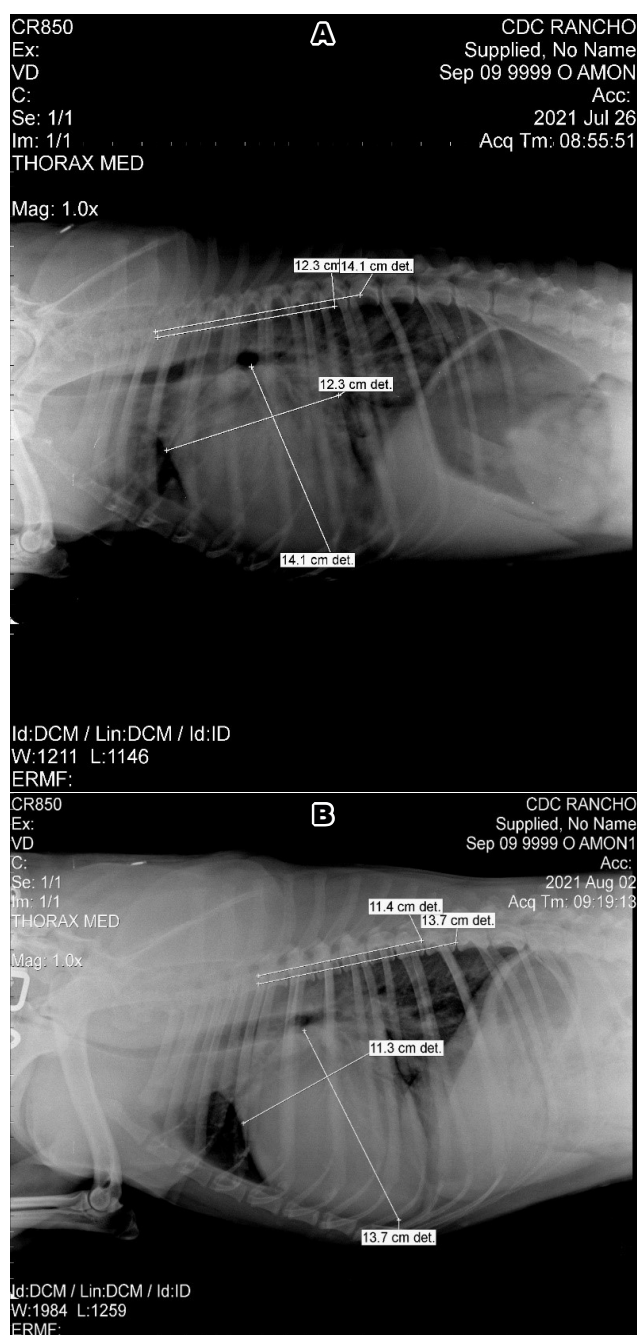


Fig. 1. Radiograph of a dog from the experimental group at the beginning of therapy (A) and on the 21st day of treatment (B). Measurement of the VHS

Table 1. Comparative assessment of radiographic changes in animals of the control group (n=6) and experimental groups (n=6), M±SD

Measurement	Control	ST		STH		References value
		Day 1 (M±SD)	Day 21 (M±SD)	Day 1 (M±SD)	Day 21 (M±SD)	
Vertebral Heart Score	8.8±0.3	12.1±0.7	11.2±0.7*	12.1±1.0	10.8±1.1*	8–10.5
Vertebral Left Atrial Size	1.5±0.1	2.5±0.4	2.1±0.3*	2.5±0.3	2.0±0.3*	1.4–2.2

Note. * — $P \leq 0.05$ — statistically significant difference compared to the beginning of therapy.

a reduction in the intensity of the bronchial pattern. In most animals, a transition from a “marked” bronchial enhancement to a “mild” or “moderate” one was noted.

Thus, radiographic examination made it possible to identify the initial degree of venous congestion and track positive dynamics during treatment, ultimately confirming the effectiveness of the applied therapy in reducing manifestations of pulmonary congestion in dogs with myxomatous mitral valve degeneration.

Table 1 provides a comparative assessment of radiographic changes in animals from the control group (n=6) and experimental groups (ST, STH, n=6) before and after treatment. Since the data in the ST group met the conditions of normal distribution (verified using the Shapiro-Wilk test, $P > 0.05$), a paired *t*-test was used to compare changes in the vertebral heart score between Day 1 and Day 21. As a result, a statistically significant ($P < 0.01$) decrease of 7.4 % in this parameter was observed, although it remained above the reference values (8–10.5 units) after treatment.

For the STH group, due to the non-normal distribution of data ($P < 0.05$ according to the Shapiro-Wilk test), the non-parametric Wilcoxon test was applied, which showed a statistically significant ($P < 0.05$) decrease in the vertebral heart score by 10.7 %.

The vertebral left atrial size in both groups initially exceeded the normal range (≈ 2.5). On Day 21 of treatment, it decreased by 16.0 % in the ST group and by 17.6 % in the STH group, aligning with the reference range ($P < 0.05$). This indicates reduced left atrial dilation and a decrease in both preload and afterload. Our re-

sults are consistent with findings from other studies that also report increased VLAS in dogs with MMVD at early disease stages, reflecting early left atrial dilation in response to mitral valve dysfunction [11, 12].

This is the first study to comprehensively analyze changes in vertebral heart score and vertebral left atrial size in dogs with myxomatous mitral valve degeneration under the influence of humic substances.

As a result of the electrocardiographic evaluation conducted in dogs with myxomatous mitral valve degeneration, several characteristic features were identified. The primary focus was placed on assessing the functional state of the cardiac conduction system, the nature of rhythm disturbances, and the relationship between echocardiographic parameters (particularly left atrial dilation) and ECG wave morphology.

The main objectives of ECG analysis were to detect conduction disturbances, changes in interval durations, and wave amplitudes, which could indicate structural changes in heart chambers or pathological processes in the myocardium. In the control group (n=4), ST (n=5), and STH (n=3) groups, respiratory (sinus) arrhythmia was observed (fig. 2). This is a result of parasympathetic nervous system influence on the sinoatrial node and is considered normal in dogs with high vagal tone at rest, thus requiring no additional correction.

The P wave interval and amplitude in all groups remained within reference values (up to 0.04 sec. and 0.4 mV). However, no significant differences were detected between groups, indicating a lack of pronounced treatment effect on these electrocardiographic parameters.

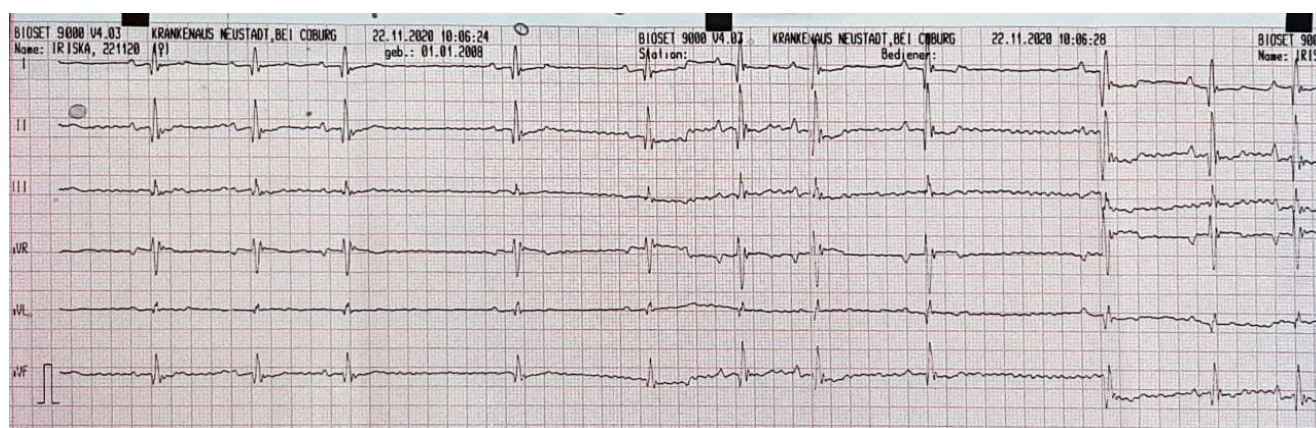
**Fig. 2.** Electrocardiogram of a dog with MMVD (Leads I, II, III, aVR, aVL, aVF; tape speed 50 mm/s). The recording shows sinus arrhythmia

Table 2. Comparative assessment of electrocardiographic results in animals of the control group (n=6) and experimental groups (n=6), M±SD

Parameters	Control	ST		STH		References value
		Day 1 (M±SD)	Day 21 (M±SD)	Day 1 (M±SD)	Day 21 (M±SD)	
P, mV	0,2±0,04	0,18±0,08	0,2±0,06	0,26±0,09	0,2±0,07	Up to 0,4
P, sec	0,03±0,01	0,04±0,01	0,03±0,01	0,04±0,01	0,03±0,01***	Up to 0,04
PQ, sec	0,08±0,02	0,09±0,02	0,07±0,02	0,11±0,05	0,09±0,03	0,06–0,13
QRS, sec	0,06±0,02	0,05±0,01	0,04±0,01	0,07±0,02	0,07±0,03	Up to 0,7
R, mV	1,1±0,3	0,9±0,2	0,9±0,2	1,15±0,5	0,8±0,2	Up to 3
QT, sec	0,16±0,05	0,18±0,02	0,17±0,02	0,17±0,06	0,15±0,03	0,15–0,24
HR, bpm	181,0±11,2	156±18,1	142±10,2*	184±9,0	172±4,2**	60–170

Note. * — $P < 0.01$; ** — $P < 0.05$ compared to Day 1, *** — $P < 0.05$, a statistically significant difference between day 1 and day 21 according to the t -test, $P = 0.025$. (Despite minimal absolute changes, significance was confirmed when analyzing individual values).

Analysis of the R waves, QRS complex, and PQ segments on the ECG showed no changes in either group at the beginning or end of the study, suggesting the absence of pathological alterations in impulse conduction during the development of this disease (table 2). In some animals, excitability disturbances were identified. These arrhythmias are commonly observed in cases of myxomatous mitral valve involvement, likely due to mechanical irritation of the endocardium by stretched chordae or hypoxia, which can prolong the refractory period in myocardial regions near the papillary muscles [10].

Radiographic studies confirm the development of cardiomegaly in dogs with myxomatous mitral valve degeneration and the presence of pulmonary congestion. During treatment, a positive trend was observed in the form of reduced heart size and normalization of pulmonary circulation, indicating the effectiveness of the prescribed therapy, particularly the use of diuretics and a comprehensive approach to correcting hemodynamic disorders.

The results of the study on electrocardiographic changes in dogs with myxomatous mitral valve degeneration demonstrated that the use of standard therapy in combination with humic substances had no effect on the electrophysiological characteristics of the heart.

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Зміни рентгенографічних та електрокардіографічних показників у собак з міксоматозною дегенерацією мітрального клапана при застосуванні гумінових речовин

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У статті наведено результати дослідження впливу гумінових речовин у собак із міксоматозною дегенерацією мітрального клапана в стадії С, які отримували стандартну терапію згідно з рекомендаціями Американського коледжу ветеринарної медицини внутрішніх органів. Проведено комплексну оцінку рентгенологічних та електрокардіографічних показників до та після лікування. Встановлено, що додавання гумінових речовин до стандартної терапії сприяло зменшенню розмірів серця та зниженню застійних явищ у легенях. Вірогідного впливу на електрокардіографічні показники не виявлено. Отримані результати свідчать про доцільність подальшого вивчення гумінових речовин як додаткового засобу у комплексному лікуванні собак із міксоматозною дегенерацією мітрального клапана.

Ключові слова: ендокардіоз, фізіологія, антиоксиданти, ветеринарна кардіологія, серцева недостатність