

## Original research

### Echocardiography evaluation of changes in the cardiac function in dog with myxomatous mitral valve disease treated with humic formulation

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**Abstract.** Myxomatous degeneration of the mitral valve, or endocardiosis, represents the most prevalent cardiovascular pathology among small- and medium-sized dog breeds. The disease is chronic, with clinical signs possibly emerging over a period of 3–4 years from the initial involvement of the mitral valve leaflets. The primary therapy initiates at stage B2, according to the ACVIM consensus which is characterized by the absence of clinical signs. In our research, animals were chosen based on a medical history indicating symptoms including episodic coughing, decreased tolerance to physical exertion, and instances of dyspnea during periods of rest or sleep. A total of 12 animals presenting with pathognomonic signs of heart disease were investigated. The diagnosis was confirmed using echocardiography, and the animals were conditionally divided into one control and two exposed to humic acids formulation groups. The results of an echocardiographic investigation regarding the functional status of the heart in diseased animals treated with bioactive substance of humic origin are provided. During the 21 days the second experimental group was administered the "Humilid" preparation at a dosage of 10 mg/kg of active substance once daily. Echocardiographic measurements were performed at the onset of the study and on the 21st day of the investigation. Obtained results demonstrated that exposure to "Humilid" with the standard therapy on the 21st day of study improves the functional parameters of the heart. Specifically, there was observed a reduction in left atrial and left ventricular dilation by 12.5% and 11.8%, respectively, although statistically insignificant ( $p > 0.05$ ). Nevertheless, the treatment with humic acids led to a decrease in severity of clinical symptoms. No effect of bioactive substances contained humic acids was observed in respect to the ejection fraction and fractional shortening. Aforementioned parameters were unaltered in both groups within the reference norms throughout the. Furthermore, no adverse or toxic impact of the humic acids formulation on the detected physiological parameters of the animals were observed. The findings from our study may serve for a comprehensive evaluation of the risk related to the development of stagnant phenomena, as well as in finding out the effects of humic substances on myocardial function and cardiac physiological properties.

**Keywords:** dogs, bioactive substances; nutritional supplements, heart failure; myxomatosis.

### Ехокардіографічна оцінка функції серця у собак з ендокардіозом мітрального клапану на тлі застосування гумінових речовин

**Анотація.** Міксоматозна дегенерація мітрального клапану або ендокардіоз є найчастішим захворюванням серцево-судинної системи серед собак дрібних та середніх порід. Захворювання хронічне, а клінічні ознаки можуть проявлятися впродовж 3–4 років з моменту першого ураження стулок мітрального клапану. Основна терапія розпочинається на стадії B2 за протоколу ACVIM, яка ще не має клінічних ознак. Для проведення наших досліджень ми відбирали тварин, у яких в анамнезі були присутні такі симптоми як: періодичний кашель, відсутність лояльності до фізичних навантажень та задихка у стані спокою або сну. Всього досліджено 12 тварин з патогномічними ознаками захворювання серця. За допомогою ехокардіографії діагноз був підтверджений, а тварин умовно розподілили на 2 дослідні групи та 1 контрольну. Наведено результати ехокардіографічних досліджень функціонального стану серця у хворих тварин на тлі застосування біологічно-активної речовини гумінової природи. Другій дослідній групі протягом 21-го дня задавали препарат «Гумілід» із розрахунку 10 мг/кг по діючій речовині 1 раз на добу. Ехокардіографічні вимірювання проводили на початку дослідження та на 21-й день дослідження. За результатами досліджень встановлено, що додавання «Гуміліду» до стандартної терапії на 21-й день дослідження викликає покращення функціональних властивостей серця, а саме зменшення дилатації лівого передсердя та лівого шлуночка на 12,5% та 11,8% відповідно, хоча є не достовірним ( $p > 0.05$ ), але це привело до зменшення вираженості клінічних ознак. Не було виявлено ефекту біологічно-активних речовин на основі гумінів на фракцію викиду та фракцію скорочення, в обох групах ці показники не виходили за межі референтних норм, як на початку дослідження так і на 21-й день. Також не було виявлено жодного побічного ефекту або негативної дії препарату на фізіологічні показники хворих тварин. Отримані нами результати можуть бути застосовані для комплексної оцінки ризиків розвитку застійних явищ так само, як і у дослідженні ефектів гумінових речовин на функціональний стан міокарду та фізіологічні властивості серця.

**Ключові слова:** собаки; біологічно-активні речовини; кормові добавки; серцева недостатність; міксоматоз.

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## Introduction

Endocardiosis or myxomatous degeneration of the mitral valve is the most common cardiovascular disease among small and medium-sized dog's breeds (Mattin et al., 2019). This pathology accompanied by the morphological changes in the valve itself and characterized by the metamorphosis of delicate and translucent leaflets into coarse and opaque structures. Besides, other abnormalities including the deformation in the entire complex of mitral valve and the chordae tendineae attachment sites. Cellular disturbances were depicted as the alterations within the spogiosa, characterized by the accumulation of proteoglycans, as well as in the fibrosa of the leaflets, where the elastin and collagen fibers undergo degradation (Rippe et al., 2012). The complex of pathological changes leads to the disruption of dynamic-mechanical behavior of the valve, characterized by leaflet displacement and improper closure during systole, resulting in the ejection of blood from the left ventricle to left atrium, commonly referred to as regurgitation. Chronic volume overload of the left atrium leads to progressive enlargement, resulting in increased pressure within the pulmonary veins. Subsequently, this pathophysiological process leads to extravasation of exudate into bronchial lumen, eliciting cough and dyspnea. The diagnosis of mitral valve endocardiosis requires the identification of a spectrum of symptoms, including signs of stagnation in the small circle of blood circulation, alongside findings from additional diagnostic modalities, such as radiography, electrocardiography and echocardiography.

Echocardiography is considered the gold standard for definitive diagnosis (Vezzosi et al., 2021). In addition to the morphological changes detection in the valve structure and cardiac chambers, it enables the assessment of the correctness of transvalvular blood flows, their velocity, and direction through the Doppler effect. Additionally, echocardiography (ECHO) is potent to detect the emergence of stagnant phenomena in both the lesser and greater circulatory systems.

The correction of the functional changes in the heart associated with this pathology is typically achieved through surgical valve replacement. However, due to the costliness of this procedure, patients are often prescribed pathogenetic therapy following a standard protocol for patient support. This typically includes pimobendan, as a phosphodiesterase 3 inhibitor, known for its positive inotropic effect and vasodilatory properties (Keene et al., 2019).

To alleviate stagnant symptoms and reduce cardiac preload the diuretics are recommended, including loop diuretics such as furosemide and aldosterone antagonist – spironolactone. The latter also exerts additional effects in reducing myocardial remodeling (Coffman et al., 2021).

Recently, the application and evidence of prescribing antioxidants for cardiovascular pathologies have become increasingly relevant. Among them, notable antioxidants include vitamins E and C, as well as beta-carotene. Studies in rodents have shown a positive

effect of vitamin E on oxidative processes in myocardium. However, in human studies, its supplementation increased the risk of heart failure and appearance of negative inotropic effects (Münzel et al., 2015; Khan et al., 2021). The supplementation of vitamin C to the diet of rodents resulted in improved positive inotropic effects on the myocardium. However, as of today, the detailed impact of vitamin C on the development and the progression of heart failure remains largely unstudied (Wannamethee et al., 2013).

Research of the beta-carotene effect on the human body and experimental animal's models, particularly in hamsters, has shown that it increases the risk of respiratory system oncological diseases and elevates the risk of mortality from cardiovascular pathologies (Middha et al., 2019; Kordiak., 2022). One of the potential protector among bioactive substances that may ameliorate endocardiosis complications in the animals are substances of humic nature. It has been demonstrated that humic substances exhibit antioxidant properties due to their phenolic groups, along with neutralization of reactive oxygen species. Furthermore, they stimulate transamination processes in the liver through alanine and pyruvate rather than aspartic acid (Dyomshyna et al., 2021). Pomic substances undergo rapid metabolism, are capable of chelation and are non-toxic to tissues. (Stepchenko et al., 2019). Additionally, they can serve as a source of new biological substances that support organismal homeostasis at any level, facilitating functional restoration under pathological or extreme conditions (Lisna & Stepchenko, 2022). However, the issue regarding the potential impact of humic substance-based medications on the functional status of the heart and organism in dogs afflicted with mixomatous degeneration of mitral valve, remains unresolved. Therefore, the study of protective effect of humic acids on the endocardiosis complication is actual point small animals health.

The aim of the presented study was to determine the protective effect of the bioactive feed supplement "Humilid" on the functional properties of the heart, during the treatment of dogs with mitral valve endocardiosis at stage "C".

## Materials and methods

The study was carried out in the Veterinary Center of Veterinary Medicine Faculty of Dnipro State Agrarian and Economic University and LLC "Veterinary Space Discovery" in Dnipro city during 2020-2022. Twelve dogs were involved into the study of the "Humilid" effect on the dogs with mitral valve myxomatous degeneration. The dogs from 8 to 12 years age were housed in domestic environments. Differentiation of severity was carried out according to the standard scheme of clinical research: collection of anamnesis, clinical examination and further carrying out of instrumental diagnostics with echocardiography analysis.

Following the American College of Veterinary Internal Medicine (ACVIM) recommendation, animals were separated according to the severity of disease progression, as follows (Table 1): stage B1-animals presenting with systolic murmurs but lacking

**Table** – General characteristics of the studied animals with stage C mitral valve endocardiosis

Animal data	Control	Group, ST	Group, ST+H
Weight, kg	9.7 ± 0.6	11.4 ± 0.7	10.9 ± 0.5
Age, years	8.5 ± 0.5	9.2 ± 0.4	9.5 ± 0.4
Sex:			
females	5	3	1
males	1	3	5
Treatment	Without treatment	Pimobendan Furosemide Spironolactone	Pimobendan Furosemide Spironolactone Humilid

clinical signs of disease; stage B2 – asymptomatic animals with notable regurgitation leading to hemodynamic and cardiac geometry changes; stage C – animals presenting with clinical signs of heart failure precipitated by mitral valve endocardiosis (Keene et al., 2019). However, due to the low number of patients with stage B1 (n = 1), B2 (n = 7) and D (n = 0), these animals were not included in the study.

The study carried out with 18 dogs of both genders. The animals with confirmed endocardiosis stage C were conditionally divided into 2 groups: the standard therapy (ST) (n = 6) and the standard therapy combined with exposure to humic compounds (ST+H) group (n = 6). The average age of the animals in the ST group was  $11.4 \pm 0.72$  years, and in the ST+H group, it was  $10.9 \pm 0.54$  years. The mean weight of all dogs was  $9.2 \pm 0.49$  kg and  $9.5 \pm 0.42$  kg respectively. The control group (C) consisted of clinically healthy animals (n = 6).

Animals of both the ST and ST+H groups were administered standard treatment according to the consensus of the American College of Internal Veterinary Medicine. This included the phosphodiesterase 3 inhibitor pimobendan, at a dosage of 0.2 mg/kg orally every 12 hours, administered on an empty stomach; the aldosterone antagonist – spironolactone 2 mg/kg orally every 12 hours; loop diuretic – furosemide at a dosage of 2 mg/kg, orally twice daily, administered on an empty stomach (Keene et al., 2019). The animals diet consisted of commercial feeds from various brands, without any additional vitamin and mineral supplements.

The design of present study was constructed accordingly the aim to clarify the effect of humic substances alongside standard therapy, animals in the ST+H group received supplementation with bioactive preparation “Humilid” in the dose of 10 mg/kg of the active substance. The supplement was administered orally once daily, i.e, every 24 hours from the initial administration. To facilitate better consumption, the supplement was pre-diluted with water at a ratio of 1:5, significantly reducing the occurrence of vomiting reflexes. The duration of the treatment for both the ST and ST+H groups was 21 days. Animals assessments for all groups were conducted on the day before treatment beginning and at 21 days post the start of therapy.

The transthoracic echocardiography was performed using the Esaote MyLab Gamma device (USA), employing a phased array transducer of 3-5 MHz and a micro-convex transducer of

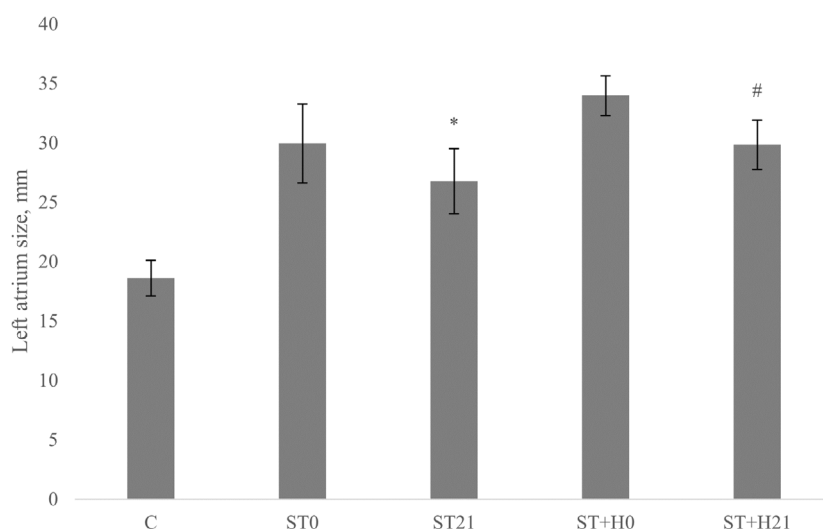
7-10 MHz. The reference ranges of parameters were calculated based on the mean body weight (Boon, 2011). The animals were put on specialized table with a thoracic cage cutout, facilitating examination in lateral recumbent positions, including both right and left sides. To improve the sonographic visualization, standard projections were employed, including: right parasternal short-axis view, right parasternal long-axis view, left parasternal long-axis view, and left parasternal apical four-chamber view along the long axis. To evaluate the cardiac condition, the following parameters were employed: morphological degeneration and uniform thickening of valves; left atrium size (LA), by millimeters; left atrial size indexed to aortic diameter (LA/Ao), unit; left ventricular internal diameter end-diastole or end-systole (LVIDd, LVIDs), in millimeters; interventricular septal thickness in systole and diastole (IVSd, IVSs), in millimeters; normalized left ventricular diameter in diastole (LVIDn), unit. The systolic and diastolic function of the left ventricle was assessed echocardiographically using the following parameters: Simpson ejection fraction (EF), % and fractional shortening (FS), %.

The data are presented as mean  $\pm$  standard error of mean ( $M \pm SEM$ ). Between-group differences in the obtained data were analyzed by one-way analysis of variance (ANOVA) with the Bonferroni post-hoc tests. The data are presented as mean  $\pm$  standard error of mean ( $M \pm SEM$ ). The differences in the analyzed data were considered reliable at  $p < 0.05$ .

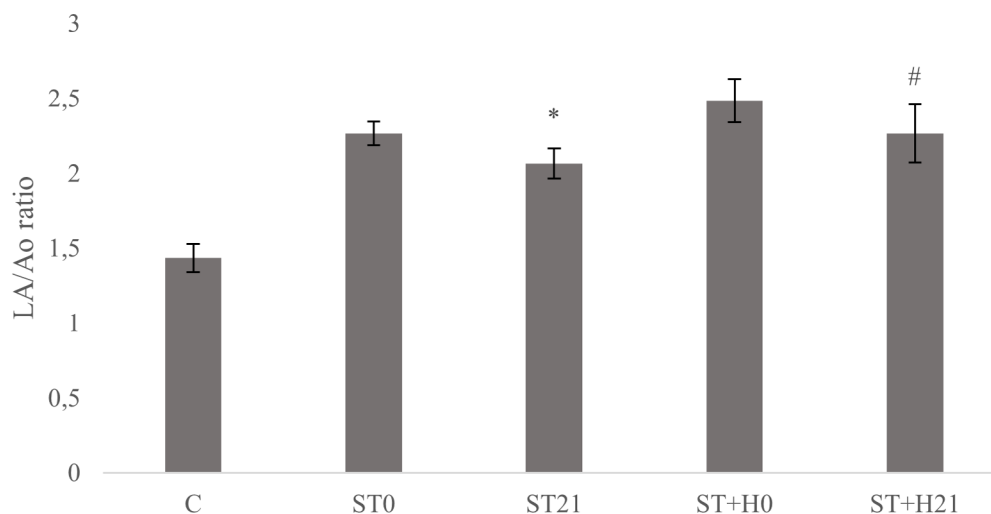
## Results

The functional and morphological status of the hearts of animals affected by mitral valve endocardiosis was investigated using echocardiography. During the initial examination, dilation of the left atrium (LA) was identified, indicating potential volume overload of left atrium.

The animals of the ST+H group received comprehensive therapy, including the administration of the “Humilid” preparation. Evaluation of the LA parameter on the 21st day of “Humilid” application revealed a positive trend in LA diameter reduction by 10.6% ( $p > 0.05$ ) and 12% ( $p < 0.15$ ), respectively, compared to the same parameter on the first day of examination in animals from both groups (Fig. 1). Despite the fact that the detected changes did not reach statistical significance, the trend of such changes suggests



**Fig. 1.** The ratio of the sizes of the left ventricle to assess the degree of left atrial dilatation. C – control group; ST-0 – group before standard therapy, ST-21 – 21 days exposed ST group; ST+H0 – group before standard therapy + humic substances, ST+H21 – 21 exposed ST+H group. Significance of the differences in compare to ST0 group: \* –  $P > 0.05$ . Significance of the differences in compare to ST+H0 group: # –  $P < 0.15$ .



**Fig. 2.** The LA/Ao ratio as a marker of the degree of left atrial dilatation. C – control group; ST-0 – group before standard therapy, ST-21 – 21 days exposed ST group; ST+H0 – group before standard therapy + humic substances, ST+H21 – 21 exposed ST+H group. Significance of the differences in compare to ST0 group: \* –  $P > 0.05$ . Significance of the differences in compare to ST+H0 group: # –  $P > 0.05$ .

a moderate protective effect of preparation on the dysfunction of the left atrium valve.

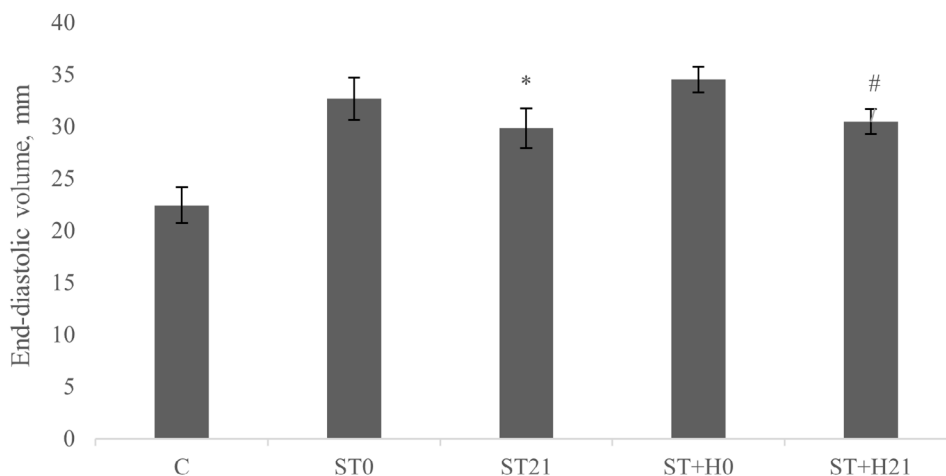
The aortic diameter (Ao) among the groups showed no significant difference and did not exceed the reference norms. The left atrium to aorta ratio (LA/Ao) was evaluated for an additional assessment of left atrial dilation. On the 21st day of the study, the LA/Ao ratio showed a slight decrease of 6.8% ( $p > 0.05$ ) in the animals from ST group and 8% ( $p > 0.05$ ) in animals of the ST+H group (Fig. 2). A positive correlation between these parameters amounted to  $r=0.86$  in the first group and  $r=0.97$  in the second, was observed. This indicates that improvements in the left atrium to aorta ratio coincided with a reduction in symptoms such as coughing and dyspnea during physical exertion.

The left ventricular internal diameter end-dyastole (LVIDd) in animals from both groups on the first day of the study was slightly

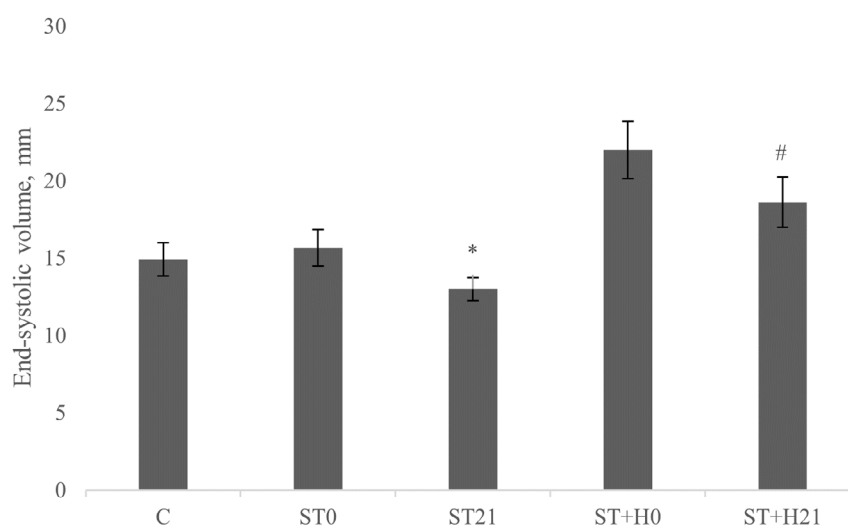
elevated compared to the reference norm by 1.5% ( $p > 0.05$ ) and 6.8% ( $p < 0.05$ ), respectively (Fig. 3). The assessment of LVIDd and left ventricular internal dimension end-systole (LVIDs) was conducted to analyze eccentric hypertrophy of the left ventricular cavity, which may occur due to increased blood volume secondary to valve apparatus lesions.

The changes in the LVIDd on the 21st day of the study were statistically non-significant in the ST group ( $p > 0.05$ ) but decreased by 8.8% while in the ST+H group, they decreased by 11.8% ( $p < 0.05$ ) compared to baseline values. This may indicate a positive therapeutic effect on chamber dilation by reducing its volume with the total blood mass.

The LVIDs decreased by 25.6% ( $p < 0.15$ ) in animals from the ST group and by 7.4% ( $p < 0.15$ ) in animals from the ST+H group, which was statistically non-significant.



**Fig. 3.** The ratio of LVIDd between groups for the assessment of dilatation of the left ventricular cavity. C – control group; ST-0 – group before standard therapy, ST-21 – 21 days exposed ST group; ST+H0 – group before standard therapy + humic substances, ST+H21 – 21 days exposed ST+H group. Significance of the differences in compare to ST0 group: \* –  $P > 0.05$ . Significance of the differences in compare to ST+H0 group: # –  $P < 0.05$ .



**Fig. 4.** The ratio of LVIDs between groups for the assessment of dilatation of the left ventricular cavity. C – control group; ST-0 – group before standard therapy, ST-21 – 21 days exposed ST group; ST+H0 – group before standard therapy + humic substances, ST+H21 – 21 exposed ST+H group. Significance of the differences in compare to ST0 group: \* –  $P < 0.15$ . Significance of the differences in compare to ST+H0 group: # –  $P < 0.15$

The normalized left ventricular internal diameter (LVIDn) during the initial examination was varied between 1.7 and 1.9 units, indicating a pathological deviation from the reference norms in both groups. An elevation in this parameter suggest secondary cardiomegaly due to mitral valve pathology (Rishniw & Brown, 2022). However, on the 21st day from the initiation of therapy, this index decreased by 11.5% ( $p > 0.05$ ) in the ST group and by 15.4% ( $p > 0.05$ ) in ST+H group (Fig. 4). Additionally, the decrease in the level of the normalized left ventricular diameter also affects the severity of the disease progression. Therefore, more pronounced changes were observed in the animals from the ST+H group, where the “Humilid” preparation was introduced as additional therapy. Such a change may indicate a positive influence of bioactive substances on the condition of myocardial muscle fibers during systole and diastole.

The functional parameters of cardiac function, namely ejection fraction (EF) and fractional shortening (FS), showed no alterations compared to the reference values in both investigated groups. However, a negative trend in FS below the lower limits of the reference norms was observed on the 21st day of the study, indicating a gradual transition from compensatory reaction to decompensation despite the added therapy.

## Discussion

Improving the functional capacity of the heart stands as the foremost priority for any veterinary physician in the treatment of animals with mitral valve endocardiosis. Given that echocardiographic examination of animals with this pathology, using humic substances is conducted for the first time. Perform an analysis of works on this topic poses a challenging task. The studies on isolated rat hearts (Lasukova et al., 2019) demonstrated that, in addition to their cardioprotective effect, humic substances positively affect inotropic action, although this was not confirmed by echocardiography. The results of recent studies have indicated that the use of inotropic agents, specifically pimobendan, effectively enhances cardiac functional capacities by augmenting both ejection fraction and fractional shortening (Boonpala et al., 2023; Sánchez et al., 2023). Additionally, our investigation aligned with the studies (Vereb et al., 2024; Franchini et al., 2021) regarding the reduction in left atrial size in the short term and demonstrated a positive impact

of the clinical condition of animals with mitral valve myxomatous degeneration. This was evidenced by a decrease in cough frequency and intensity, as well as a reduction in stagnant phenomena in the systemic circulation. The comprehensive therapy, incorporating the medication “Humilid”, induced a positive trend in which left atrial dilation was reduced in both groups relative to baseline values. The decrease in left atrial enlargement is associated with a reduction in blood volume overload and indicates a decrease in intracardiac pressure, thereby improving the induction of stagnant phenomena in the systemic circulation. Thus, the presented cardioprotective effect of humic compounds on stagnant phenomena in the systemic circulation is correlated with the effects of other inotropic agents.

The determined changes in the left atrium to aorta (LA/Ao) ratio in both groups, as observed in our study, did not align with the established reference norms ( $< 1.6$  unit). On the first day of the study, this indicator corresponded to  $2.2 \pm 0.2$  units in the ST group and  $2.06 \pm 0.2$  units for 21 days. Meanwhile, in the ST+H group, it measured  $2.5 \pm 0.3$  units and  $2.3 \pm 0.4$  units, respectively. However, the observed trend indicates a positive effect regarding the suppression of the left atrial pathogenesis. The study results also allowed for an assessment of the correlation between the level of left atrial pathogenesis development and clinical data of affected animals, which closely aligns with findings from (Boswood et al., 2016) regarding the extension of the preclinical period in affected animals.

The left ventricle internal end-systolic (LVIDs) and end-diastolic (LVIDd) serve as indicators of chamber dilation due to volume overload and correlate with the severity of the condition (Donghyun et al., 2018; Grosso et al., 2023). In our study, we detected minor dilation of the left ventricular cavity in animals from ST group, measuring  $32.7 \pm 5.6$  mm, and  $31.8 \pm 2.8$  mm in the ST+H group. The extent of dilation decreased to within reference intervals (20.6-31.3 mm) under the influence of comprehensive therapy. However, a notable correlation with the severity of the animals condition was not identified, which may be associated with the absence of significant clinical signs or a limited sample size of the study animals.

The results of the presented study did not demonstrate significant alterations in the function of the left ventricle and left atrium throughout the 21st day. Conversely, the clinical parameters identified suggest an improvement in terms of reduced disease

manifestations. These findings could be valuable for selecting comprehensive therapy and potentially reflect the positive influence of humic substances and pimobendan on the cardiac functional status. Although in the long term, deterioration in the mechanical function of the valvular apparatus may lead to unreliable data regarding positive or negative functional changes in the heart during the use of bioactive substances. Moreover, more prolonged study (exceeding 6 months) is necessary for a more comprehensive investigation of the impact of humic substances on cardiac functional parameters since, according to (Sarcinella et al., 2019), even a six-month study interval is considered short for assessing negative trends in cardiac function.

It is noteworthy that the findings of carried out study did not demonstrate any adverse effects on alterations in the left ventricular and left atrial function, nor did they exacerbate clinical symptoms. In some animals, administration of the “Humilid” solution elicited a vomiting reflex, attributed to the sharp and bitter taste of the substance. The solution of this problem entailed increasing the dilution of the concentrated solution with distilled water at a ratio of 1:5. However, the modification feed additives with new approach based on the molecular interaction of blended short chain fatty acids with intestinal epithelium cells proposed as prospective strategy in animal health maintenance (Masiuk et al., 2022).

According to the results of NCBI PubMed database analysis, the data obtained in our study, represent the first description of the effect of humic substances on the inotropic parameters, cardiac chamber dilation, and reduction of clinical manifestations in dogs with clinical stage (ACVIM Stage C) mitral valve endocardiosis. The study of cellular disturbance as well as repair mechanisms require the application of molecular markers to characterize detailed pathways which involved into tissue-specific response against cardiovascular disorders (Faraj et al., 2022). Special interest attracts the study of the modulation and redirecting of programmed cell death to support cell viability and stable tissue functioning (Masiuk et al., 2024). Taking into the account that cardiovascular abnormality is dependent on various cell types disturbance, specific molecular markers are promising tool to find out the protective effects of bioactive compounds including humic acids.

### Conclusion

Humic compounds demonstrate both a bioactive and protective effect on functional parameters of cardiac activity. Despite the moderate effect, wherein the studied parameters were only partially improved and did not achieve the reference norm level. However, positive trend in cardiac function restoration was observed. The established correlations between the left atrium to aorta and normalized left ventricular internal diameter allowed the assessment of degree of left atrial dilation in diseased animals. No effect of humic compounds on the ejection fraction and fractional shortening which are responsible for the hearts primary functional load was observed. Thus, the impact of these effects of humic compounds in our study is not certain. Further study of these effects remains suitable to elucidate the progressive prevalence of cardiovascular disorders. The advance in the molecular marker applications is required for the treatment of mitral valve endocardiosis. The observed results may serve as prognostic markers to assess the risks of developing stagnant phenomena, which are a primary concern during therapy administration.

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### Conflict of interest

Authors declare no conflict of interests with respect to this paper.

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