https://doi.org/10.31925/farmacia.2025.1.13 **ORIGINAL ARTICLE**

CHARACTERISATION AND VALIDATION OF AN ISOPROTERENOL-INDUCED HEART FAILURE MOUSE MODEL

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Manuscript received: November 2024

Abstract

The objective of this study was to establish and characterise a mouse model of HF using isoproterenol (ISO), a non-selective beta-adrenergic agonist. Different doses of ISO, administered over a variable period (160 mg/kg, 1 day; 80 mg/kg, 5 days; 40 mg/kg, 7 days; and 20 mg/kg, 25 days) were used to obtain a reproducible and robust HF model. Echocardiographic parameters such as left ventricular anterior/posterior wall thickness in diastole/systole (LVAWd, LVAWs, LVPWd, LVPWs), left ventricle internal diameter in diastole/systole (LVIDd, LVIDs), left ventricular end-diastolic/systolic volume (LVEDV, LVESV), fractional shortening (FS), ejection fraction (EF), left ventricular mass (LV mass), relative wall thickness (RWT), stroke volume (SV), as well as histopathological analysis, were used to characterize the HF model. The validated HF mouse model will be used for pharmacological studies of novel therapeutic candidates for HF.

Rezumat

Scopul acestui studiu a fost de a realiza și caracteriza un model de IC indus la șoarece, utilizând isoproterenolul (ISI), un agonist beta-adrenergic non-selectiv. Pentru a obține un model reproductibil și robust de IC, au fost testate doze diferite de ISO, care au fost administrate pe o perioadă variabilă de timp (160 mg/kg, 1 zi; 80 mg/kg, 5 zile; 40 mg/kg, 7 zile; and 20 mg/kg, 25 zile). Pentru a caracteriza modelul de IC s-au utilizat o serie de parametri ecocardiografici, și anume grosimea peretelui anterior/posterior al ventriculului stâng în diastolă/sistolă (LVAWd, LVAWs, LVPWd, LVPWs), diametrul intern al ventriculului stâng în diastolă/sistolă (LVIDd, LVIDs), volumul ventriculului stâng la finalul diastolei/sistolei (LVEDV, LVESV), fracția de scurtare (FS), fracția de ejecție (EF), masa ventriculului stâng (LV mass), grosimea relativă a peretelui (RWT), volumul-bătaie (SV), precum și analiza histopatologică. Modelul validat de IC indus la șoarece va fi utilizat în experimente farmacologice ce includ noi candidați terapeutici pentru tratamentul IC.

Keywords: heart failure, mouse, isoproterenol, echocardiography, histopathology

Introduction

Heart failure (HF), which affects over 64 million individuals globally, is a debilitating clinical syndrome. It is characterised by clinical manifestations such as dyspnoea, orthopnea, abnormal pulmonary sounds, oedema, exercise intolerance, and jugular vein distension. These symptoms indicate the body's compensatory responses to compensate for the reduced cardiac output, whether at rest or during physical activity [7, 18].

HF results from structural and functional abnormalities affecting the two cardiac phases, the filling phase (diastole) and the emptying phase (systole). In diastole, the heart fills with oxygenated blood from the lungs, while in systole, the heart contracts and pumps the blood to the arteries. It is known that 2/3 of HF cases are due to different chronic conditions, such as ischemic heart disease, uncontrolled elevated blood pressure, valvular heart disease, and genetic or inherited cardiomyopathies [18].

Depending on the left ventricular (LV) ejection fraction (EF) value, HF is subclassified into HF with preserved EF (HFpEF, LVEF ≥ 50%), HF with mildly reduced EF (HFmrEF, LVEF 41-49%) and HF with reduced EF (HFrEF: LVEF ≤ 40%). More than 50% of patients diagnosed with HF have HFpEF; alarmingly, the 5-year mortality rate for these patients exceeds 50%, often as a result of the

numerous comorbidities frequently associated with HFpEF [7].

Regarding HFrEF, significant therapeutic advancements have been made in the past decade, including the combination angiotensin receptor-neprilysin inhibitor (*e.g.* sacubitril-valsartan), sodium-glucose cotransporter-2 (SGLT2) inhibitors, alongside the use of beta-blockers, and mineralocorticoid receptor antagonists in quadruple therapy, which has markedly reduced mortality and improved the quality of life for this category [6, 20]. However, there is a significant unmet need for therapies that improve clinical outcomes in HFpEF and HFmrEF, considering the heterogeneous clinical profile characteristic of these patients [6].

For a better understanding of the causes and aetiology of HF and for the development of new innovative therapeutic strategies, different HF animal models are used in experimental pharmacology [16]. Although the heart muscle structure of large animals (pigs, dogs, sheep) is more similar to that of humans, the HF models in smaller animals (such as mice, rats, and guinea pigs) are more commonly used [13, 14]. Among small animals, mice are the most used due to their high genetic similarity to humans; approximately 85% of mice's genes encoding protein synthesis are identical to those in the human genome. In addition, mice can be easily genetically modified, have a short reproductive cycle, and have relatively low costs for experiments [12, 15]. Thus, several mouse models of HF have been developed through a combination of genetic modifications, administration of pharmacological agents, and/or various surgical techniques [12].

One of the pharmacological HF models is based on adrenergic stimulation, using non-selective beta-adrenergic agonists (e.g. isoproterenol - ISO), alpha-adrenergic agonists (e.g. phenylephrine), alcohol or doxorubicin (DOX) [1, 10, 16]. ISO is commonly used to reproduce cardiac dysfunction and remodelling through persistent oxidative stress due to beta-adrenergic stimulation [11]. ISO stimulates cardiac beta-2 receptors, which in turn causes vasodilation with a decrease in diastolic blood pressure, leading to coronary hypoperfusion with left ventricular dysfunction and dilation [13]. At the same time, the activation of cardiac beta-2 receptors induces the transformation of cardiac fibroblasts (which represent approximately 60% of the total number of cardiac cells and approximately 15% of the total mass of cardiac cells) into active myofibroblasts, which causes an increase in the expression of extracellular matrix (ECM) components (collagen, laminin, fibronectin) [21].

The literature indicates that various doses of ISO, ranging from 5 mg/kg to 250 mg/kg, have been used over periods of 3 days to 4 weeks to induce heart failure [5, 8]. Furthermore, multiple administration methods have been reported, including intraperitoneal (i.p.) and subcutaneous (s.c.) injections, oral gavage, the use of osmotic pumps, and different strains of mice and rats [4, 22].

This study aimed to establish a reproducible mouse model for investigating heart failure. To achieve this goal, the effects of different doses of ISO administered over a variable period of time (160 mg/kg, 1 day; 80 mg/kg, 5 days; 40 mg/kg, 7 days; and 20 mg/kg, 25 days) on heart function were tested, and the results are reported here.

Materials and Methods

Animals and Experimental Design

The experiment used a C57BL/6 mouse strain, 8 - 9 weeks old, weighing 20 - 25 g (Victor Babeș Institute, Bucharest, Romania). The animals were housed at Advanced Research and Development Center for Experimental Medicine (CEMEX) in standard conditions (constant temperature of 25 ± 2°C, relative humidity of 50 ± 15% and 12 h dark/light cycle), receiving food and water *ad libitum*. The experiment was undertaken with the approval of the Research Ethics Committee of "Grigore T. Popa" University of Medicine and Pharmacy. After 7 days of accommodation, the animals were randomly allocated into five groups, each consisting of 8 mice, as follows: group 1 (G1: IS0 160): mice received 160 mg/kg of ISO in one dose; group 2 (G2: ISO 80): mice received 80 mg/kg of ISO for five consecutive days; group 3 (ISO 40): mice received 40 mg/kg of ISO for 7 consecutive days; group 4 (ISO 20): mice received 20 mg/kg of ISO for 25 consecutive days; group 5 (G5: Control): mice received 0.9% saline (NaCl) (s.c.) for 25 consecutive days. The corresponding doses of ISO were administered s.c. as 0.9% saline (0.1 mL/10 g). The animals were monitored for 25 days, and during this time, the heart function was evaluated using echocardiography. In addition, cardiomyocyte damage and fibrosis were assessed as key indicators of cardiac function *via* histopathological analysis.

The experimental protocol regarding the dose of ISO and treatment duration is justified by the absence of a validated mouse model of ISO-induced heart failure. The literature reports various studies in which ISO doses range from 5 mg/kg to 250 mg/kg, with administration times varying from 3 days to 4 weeks [5, 8].

Echocardiographic assessment

Mice were anesthetised with 1.5% isoflurane, after which they were placed in the supine position and slightly tilted to the right (at an angle of 30°). After removing the hair from the sternal area, an optimal amount of gel was applied, and then the ultrasound probe was placed on the left side. Visual assessment of global cardiac function was performed in B-mode, systolic function measurements in M-mode, while diastolic function was measured using pulsed Doppler

(PW) mode. The evaluation was conducted at two-time points: at the midpoint of the experiment (day 12 - D12) and at the end of the study (day 25 - D25), using the Visual Sonics Vevo 2100 Micro-Ultrasounds equipment (Toronto, Ontario, Canada) [13]. Cardiac function was analysed in two echocardiographic planes: PLAX (parasternal long axis view) and PSAX (parasternal short axis view), measuring [20]. Left ventricular anterior wall thickness in diastole (LVAWd), left ventricular anterior wall thickness in systole (LVAWs), left ventricular posterior wall thickness in diastole (LVPWd), left ventricular posterior wall thickness in systole (LVPWs), left ventricle internal diameter in diastole (LVIDd), left ventricle internal diameter in systole (LVIDs). Based on LVAWd, LVAWs, LVPWd, LVPWs, LVIDd, and LVIDs, the following parameters were calculated [9]: left ventricular end-diastolic volume): LVEDV = [7/(2.4 + LVIDd)] x $LVIDd^3$; left ventricular end-systolic volume): LVESV = [7/(2.4 + LVIDs)] x $LVIDs^3$; fractional shortening: FS = (LVIDd - LVIDs) / LVIDd x 100; ejection fraction: EF = (LVEDV - LVESV) / LVEDV x 100); left ventricular mass: LV mass = 1.04 x [$(LVIDd + LVAWd + LVPWd)^3$ - $LVIDd^3$] x 0.8 + 0.6; relative wall thickness: RWT = (LVPWd + LVAWd) / LVIDd; stroke volume: SV = LVEDV – LVESV.

Histopathological evaluation

At the end of the experiment (D25), the animals were sacrificed under anaesthesia with sodium pentobarbital 0.2% (i.p. 60 mg/kg), the thoracic cavity was opened, and the heart detached [3]. The histopathological exam was performed in the Histology Laboratory – Department of Morphofunctional Sciences I. The sampled hearts were fixed in 10% formaldehyde and processed according to the standard protocol for obtaining paraffin blocks. Tissue sections were obtained by cutting at 4 µm, which were spread on slides and stained with the usual Hematoxylin–Eosin (HE) stain [17]. The histological specimens were scanned using the Aperio Leica AT2 scanner (Leica Biosystems, Germany), and the digitised images were examined using the facilities of the integrated module for image analysis and processing (Aperio Scan Scope Console, Leica Biosystems, Germany).

Statistical analysis

SPSS version 19 was used to evaluate the results presented as mean ± SD (standard deviation). Data were statistically analysed using a univariate ANOVA test, and a P value of 0.05 was considered statistically significant.

Results and Discussion

Tables I and II present the echocardiographic evaluation results for both the PLAX and PSAX views at days 15 and 25 for the studied groups.

The analysis of the results revealed that the impairment of cardiac function depends on both the dosage of ISO and the duration of administration. The lowest values of EF, a relevant parameter of cardiac injury, were observed in G4 (ISO 20 mg/kg, 25 days), reaching 41.53% (PLAX) and 42.69% (PSAX), at the end of the experiment, while the values recorded for the control group (G5) were 60.66% (PLAX) and 64.94% (PSAX). In similar conditions, at the end of the experiment, the EF values recorded in the PSAX view for the other groups were 55.25% (G1), 49.27% (G2) and 50.62% (G3), respectively. This finding is further reinforced by the FS values, which were also lowest in G4, at 19.95% (PLAX) and 20.76% (PSAX).

When comparing the EF and FS values among the ISO groups (G1-G4), the most notable differences were found between G1 (ISO 160) and G2-4 (ISO 80, ISO 40, ISO 20). Although the differences among G2-4 were not significant, the ISO dose of 20 mg/kg administered for 25 days (G4) was more favourable, as no mortality was observed during the experiment. In contrast, for G1-G3, a significant mortality rate of 40-50% was noted.

The detrimental effect of ISO on cardiac function is further supported by additional echocardiographic parameters. For instance, the LV mass values in the ISO groups were considerably higher than in the control group. In the PSAX view, the LV mass in G5 was recorded at 70.60 mg, while in the ISO groups (G1-4), it was around 100 mg, indicating an increase of approximately 40%. In the case of G4, the LV mass value was 105.47 mg (PLAX)/105.35 mg (PSAX), much higher compared to the value recorded for the control (G5), 79.64 (PLAX)/70.60 (PSAX). These findings indicate that ISO induces cardiac hypertrophy, with the severity depending on the dose and duration of administration.

In addition, RWT and SV parameters were also affected in the ISO groups compared to the control group, which supports the impairment of cardiac function induced by ISO. The values recorded for G4 were 0.52 (PLAX)/0.41 (PSAX) for RWT and 24.24 (PLAX)/31.69 (PSAX) for SV. In similar conditions, the values recorded for control (G5) were 0.69 (PLAX)/0.55 (PSAX) for RWT and 19.56 (PLAX)/26.74 (PSAX) for SV.

The results of a histopathological study of the specimens from the studied groups (G1-4) and control (G5) are presented in Figures 2 - 6.

Table I

Echocardiographic parameters recorded on the 12th day of the experiment

Parameter		Groups				
		G1 (ISO 160)	G2 (ISO 80)	G3 (ISO 40)	G4 (ISO 20)	G5 (Control)
LVAWd	PLAX	0.8915 ± 0.1838	0.9603 ± 0.0493*	0.8515 ± 0.0096	0.8772 ± 0.0759*	1.0324 ± 0.0098
	PSAX	0.8205 ± 0.1429	0.8525 ± 0.0087*	0.7017 ± 0.0762*	0.8808 ± 0.0942	0.9755 ± 0.0495
LVAWs	PLAX	1.2137 ± 0.1406	1.2436 ± 0.1273	1.0560 ± 0.0653*	1.1617 ± 0.1019*	1.4002 ± 0.0196
	PSAX	1.1230 ± 0.1969*	1.3523 ± 0.0930	0.9296 ± 0.0960*	1.1519 ± 0.1607*	1.4450 ± 0.0149
LVPWd	PLAX	0.8612 ± 0.1451	0.8705 ± 0.0477*	0.9050 ± 0.0362*	0.8685 ± 0.0313*	0.6871 ± 0.0632
	PSAX	0.8846 ± 0.0662*	0.8184 ± 0.1402*	0.7790 ± 0.0142	0.8238 ± 0.0844*	0.5937 ± 0.0128
LVPWs	PLAX	1.0524 ± 0.1501	1.1068 ± 0.0271*	1.0823 ± 0.0821	1.0159 ± 0.0376	1.0046 ± 0.0252
	PSAX	1.2192 ± 0.1067	1.1590 ± 0.1165*	1.0566 ± 0.0102*	1.0571 ± 0.0748	0.9437 ± 0.0480
LVIDd	PLAX	3.4116 ± 0.6449	3.4365 ± 0.1524	3.6590 ± 0.2688	3.7099 ± 0.1640*	3.3777 ± 0.1031
	PSAX	3.7572 ± 0.39931	4.2000 ± 0.0516*	4.1491 ± 0.1720*	3.9824 ± 0.3323*	3.5083 ± 0.0125
LVIDs	PLAX	2.4895 ± 0.5757	2.5583 ± 0.2472	2.8433 ± 0.3659	3.0055 ± 0.1482*	2.4471 ± 0.1224
	PSAX	2.7660 ± 0.5493	2.9585 ± 0.1979	3.1365 ± 0.2948*	3.1785 ± 0.1960*	2.4670 ± 0.0324
LVEDV	PLAX	49.7785 ± 21.1568	49.1432 ± 5.1611	56.6301 ± 9.8863	58.9542 ± 6.0241*	49.8507 ± 0.1976
	PSAX	61.0849 ± 15.1270	78.7367 ± 2.2830	76.1046 ± 7.2313*	69.9036 ± 14.1888	50.7880 ± 0.3077
LVESV	PLAX	23.5877 ± 12.0924	24.1061 ± 5.6768	30.8463 ± 9.4991	35.4989 ± 4.1973*	18.5528 ± 0.0932
	PSAX	30.0207 ± 13.2092	33.4722 ± 5.5547*	40.6716 ± 8.7740*	40.5115 ± 6.2827*	18.8873 ± 0.1890
EF	PLAX	54.3942 ± 6.2199	51.1441 ± 6.4815*	47.0461 ± 7.5615*	39.8183 ± 3.4925*	62.9087 ± 0.1711
	PSAX	52.5368 ± 11.5605	56.6467 ± 8.2305	47.5318 ± 6.7134*	41.5163 ± 3.5890*	63.6627 ± 0.2891
FS	PLAX	27.4823 ± 3.5941	25.5678 ± 3.9480*	23.1825 ± 4.3193*	18.9873 ± 1.9400*	33.5024 ± 0.4419
	PSAX	26.8461 ± 7.4498	29.8056 ± 5.5296	23.5160 ± 3.9871*	20.0518 ± 2.1205*	34.5237 ± 0.1792
LV mass	PLAX	81.0647 ± 11.2079	86.3375 ± 11.8549	92.5945 ± 9.3008*	92.9044 ± 5.0142*	76.7573 ± 0.2179
	PSAX	91.8398 ± 5.9510	107.9502 ± 9.8412*	89.8956 ± 0.3778	100.8507 ± 8.2985*	78.5357 ± 0.2194
RWT	PLAX	0.5412 ± 0.2122	0.5140 ± 0.0102*	0.4873 ± 0.0427	0.4760 ± 0.0454	0.4769 ± 0.0188
	PSAX	0.4607 ± 0.0882	0.3731 ± 0.0640	0.3631 ± 0.0360	0.4325 ± 0.0660	0.4074 ± 0.0122
SV	PLAX	26.1908 ± 9.1073	25.2185 ± 0.5531	25.8825 ± 0.3843	23.4553 ± 3.1802*	31.4349 ± 0.3780
	PSAX	31.0642 ± 2.8187	45.4091 ± 7.8312	35.5417 ± 1.5393	29.3921 ± 8.0899	33.5757 ± 0.2353

* p <0.05 in reference to Control (G5)

Table II

Echocardiographic parameters recorded on the 25th day of the experiment

Parameter		Groups				
		G1 (ISO 160)	G2 (ISO 80)	G3 (ISO 40)	G4 (ISO 20)	G5 (Control)
LVAWd	PLAX	0.9089± 0.1511	0.8468±0.0942*	0.7410±0.1337*	0.9523±0.1203	1.0431±0.0090
	PSAX	0.8721±0.0475*	0.8833±0.0626	0.7763±0.0589*	0.8900±0.0439	0.9817±0.0361
LVAWs	PLAX	1.2419±0.2382	1.1206±0.0543*	1.0745±0.1046*	1.2182±0.1113	1.4330±0.0241
	PSAX	1.1256±0.1026*	1.2099±0.0685*	1.0859±0.0620*	1.1291±0.0309*	1.4936±0.0091
LVPWd	PLAX	0.9325±0.1361	0.8057±0.0825*	0.7705±0.1145*	0.9588±0.0818	0.9942±0.0395
	PSAX	0.8458±0.0211*	0.7819±0.0179	0.8353±0.0608	0.8055±0.0413	0.7590±0.0446
LVPWs	PLAX	1.1827±0.2161	0.9653±0.0696*	0.8908±0.1655*	1.1584±0.1255	1.3092±0.0143
	PSAX	1.2042±0.1035	1.1315±0.0578	1.1323±0.1032	1.1132±0.0504	1.0727±0.0165
LVIDd	PLAX	3.5522±0.5574	3.7451±0.0715	3.8345±0.4754*	3.6941±0.2432*	2.8970±0.0255
	PSAX	3.9151±0.2748	4.0465±0.0051	4.3824±0.0500	4.0943±0.1891*	3.2807±0.0689
LVIDs	PLAX	2.5798±0.6732	2.9681±0.0457	3.2473±0.5725*	2.9566±0.2046*	2.0773±0.0512
	PSAX	2.7979±0.1088*	3.0457±0.0953*	3.2580±0.3152*	3.2438±0.1675*	2.1625±0.0655
LVEDV	PLAX	54.1908±18.9840	60.3905±2.6593	65.7517±18.8318*	58.2596±8.8001*	33.0160±0.0738
	PSAX	66.9766±11.1009*	71.8474±0.0590	87.1048±2.3648	74.2043±8.1220*	41.5325±0.2496
LVESV	PLAX	26.1348±14.7805	34.5223±1.2012	44.3717±18.0292*	34.0152±5.6144*	13.3512±0.1935
	PSAX	29.7883±2.8259*	36.1366±2.7666*	43.1617±10.1273*	42.5100±5.4589*	14.6380±0.2210
EF	PLAX	54.8610±13.0871	42.8330±4.5573*	35.3208±8.9420*	41.5301±3.6618*	60.6611±0.5379
	PSAX	55.2503±3.6723*	49.2729±3.8537*	50.6252±10.1132	42.6955±3.1801*	64.9392±0.1448
FS	PLAX	28.2422±8.2721	20.7776±2.6265*	16.6886±4.6144*	19.9360±2.0935*	30.9825±0.1070
	PSAX	28.4515±2.5481*	24.8774±2.3492*	26.0238±6.2861	20.7655±1.8447*	34.6766±0.2219
LV mass	PLAX	92.6190±3.3785*	88.5476±10.2069	80.7633±1.3387	105.4764±14.9832	79.6476±0.1727
	PSAX	100.0560±14.2295*	102.0484±5.1260*	109.3835±2.4099	105.3575±11.7710*	70.6029±0.2768
RWT	PLAX	0.5388±0.1788	0.4464±0.0551*	0.4360±0.1234*	0.5208±0.0744*	0.6935±0.0085
	PSAX	0.4408±0.0240*	0.4147±0.0154*	0.3655±0.0034*	0.4148±0.0214*	0.5526±0.0274
SV	PLAX	28.0560±5.0185*	26.0029±3.8498*	21.4727±0.8201*	24.2445±4.1828	19.5661±0.2237
	PSAX	37.1883±8.6315	35.7098±2.8232*	43.8552±7.7622*	31.6943±4.0699	26.7462±0.1110

* p <0.05 in reference to Control (G5)

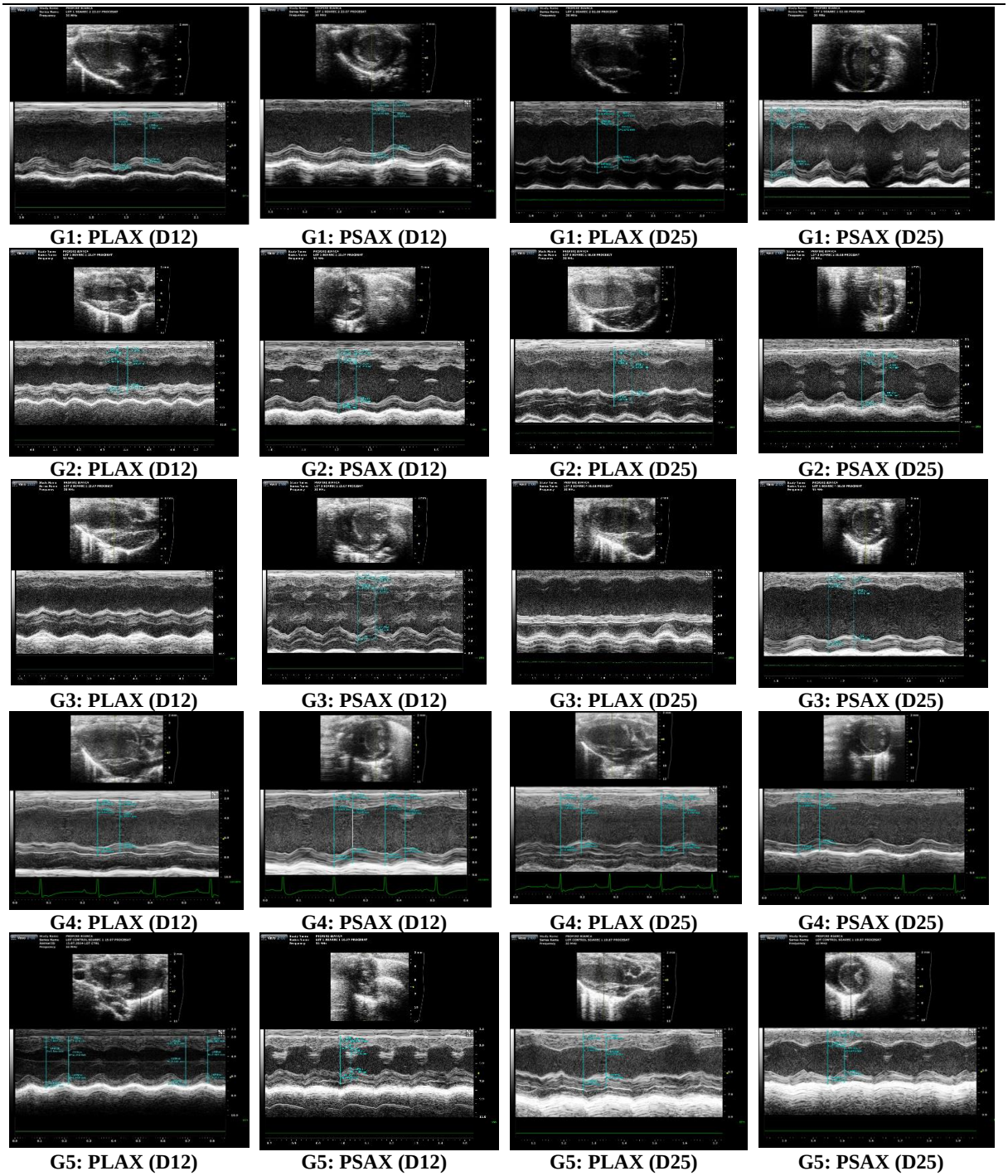

Figure 1.
Echocardiographic images (PLAX, PSAX views) for studied groups (G1: ISO 160; G2: ISO 80; G3:ISO 40; G4:ISO 20; G5: Control), recorded on 12th day and 25th day of the experiment

In the case of G1 (ISO 180), the histological examination of the heart fragments revealed morphological changes in the inner third of the myocardium. In this area, some cardiomyocytes showed degenerative lesions, such as hypereosinophilic cytoplasm, loss of double striation, and isolated aspects of hyalinisation. Associated, a chronic inflammatory infiltrate associating acute-type cellular elements (PMNn), focally located, dissociating normal and modified cardiomyocytes, with slight peri-inflammatory fibrosis – but without extension into the inter-cardiomyocyte space (Figure 2a, b). Additionally, in some specimens, small calcifications located in a limited area of fibrosis that also included some cardiomyocytes with small sizes and degenerative lesions were also identified in the inner third of the myocardium (Figure 2c, d).

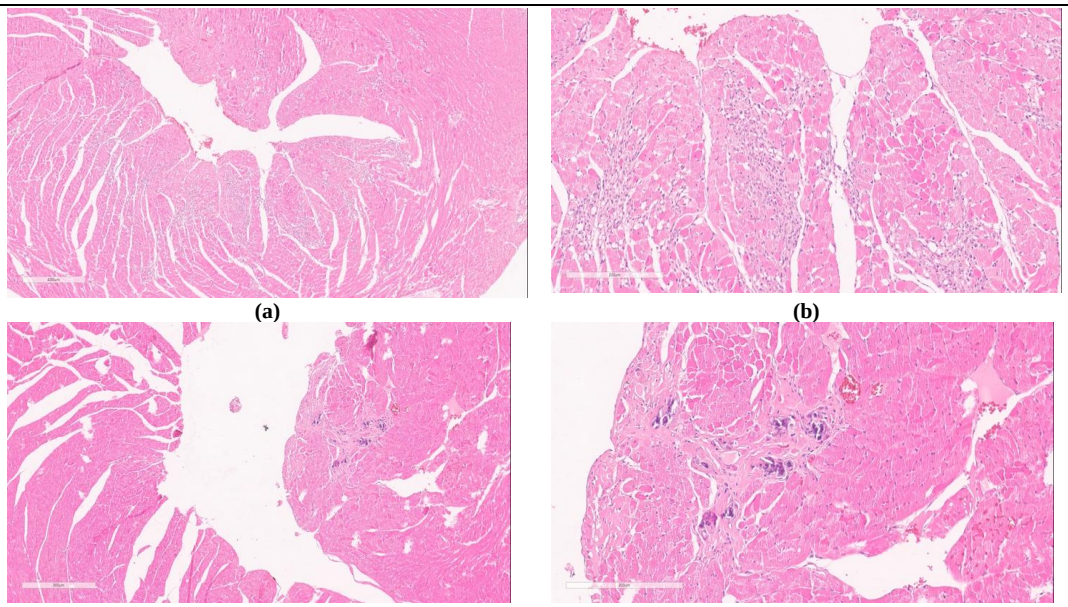

Figure 2.
Cross-section through the heart (G1, D25), a: moderate inflammatory infiltrate present in the inner third of the myocardium, b: moderate inflammatory infiltrate, focal myocytes with degenerative changes, c: isolated microcalcifications in the inner third of the myocardium, d: detail for microcalcifications surrounded by a reduced area of fibrosis

For G2 (ISO 80), the myocardial inflammatory infiltrate showed similar characteristics to those observed in G1. In addition, the accumulation of macrophages loaded with lipofuscin pigment (wear pigment) was also identified in the valvular connective tissue, suggesting degenerative lesions (Figure 3a, b).

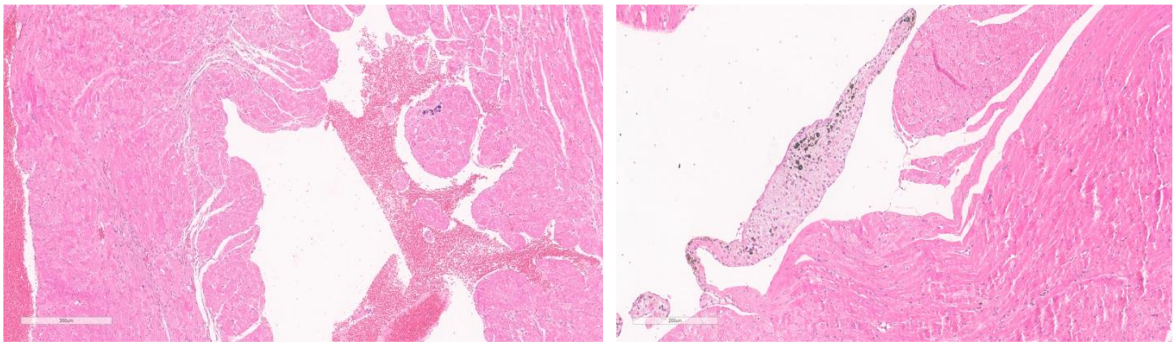

Figure 3.
Cross-section through the heart (G2, D25), a: minimal inflammatory infiltrate dispersed in the myocardium, b: valvular structure showing in the connective axis macrophages loaded with lipofuscin pigment

For the specimens belonging to G3 (ISO 40), the following characteristics were noted: the focal presence, throughout the thickness of the myocardium, of some cardiomyocytes with degenerative lesions: hypereosinophilic cytoplasm, with the loss of double striation and isolated aspects of hyalinisation; reduction of the inflammatory component up to complete absence, with the consequent development of minimal fibrosis at the level of the myocardium; the identification, in some specimens, in the inner third of the myocardium, of an extensive area (approximately 20% of the circumference) with central necrotic lesions surrounded by a wide area of fibrous connective tissue (Figure 4a, b, c, d).

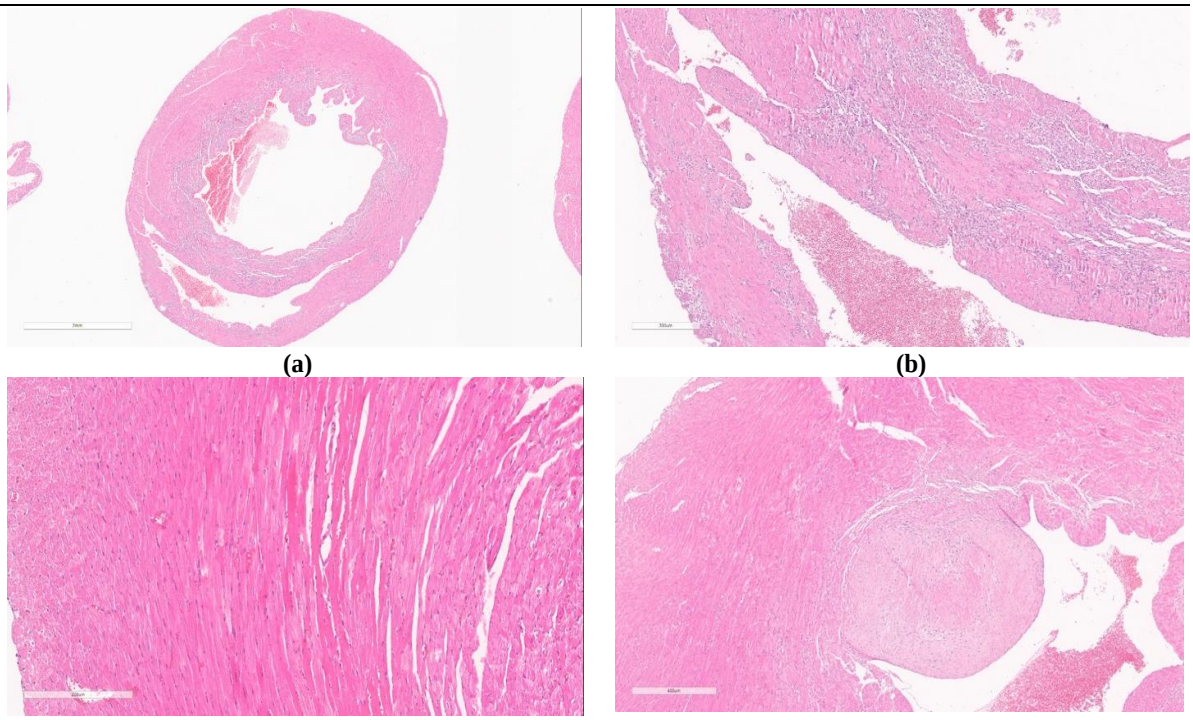

Figure 4.
Cross-section through the heart (G3, D25), a: well-represented inflammatory infiltrate, located circumferentially in the inner third of the myocardium, b: detail for the well-represented inflammatory infiltrate, localized throughout the thickness of the myocardium, c: rare cardiomyocytes with degenerative changes, d: central necrosis area surrounded by a wide area of fibrous connective tissue

For G4 (ISO 20), numerous cardiomyocytes showed similar degenerative changes to those present in G3 - namely, intense eosinophilic cytoplasm without double striation and a hyaline appearance; these lesions were widespread within the myocardium and were associated with fibrous connective tissue forming narrow strips (Figure 5a, b).

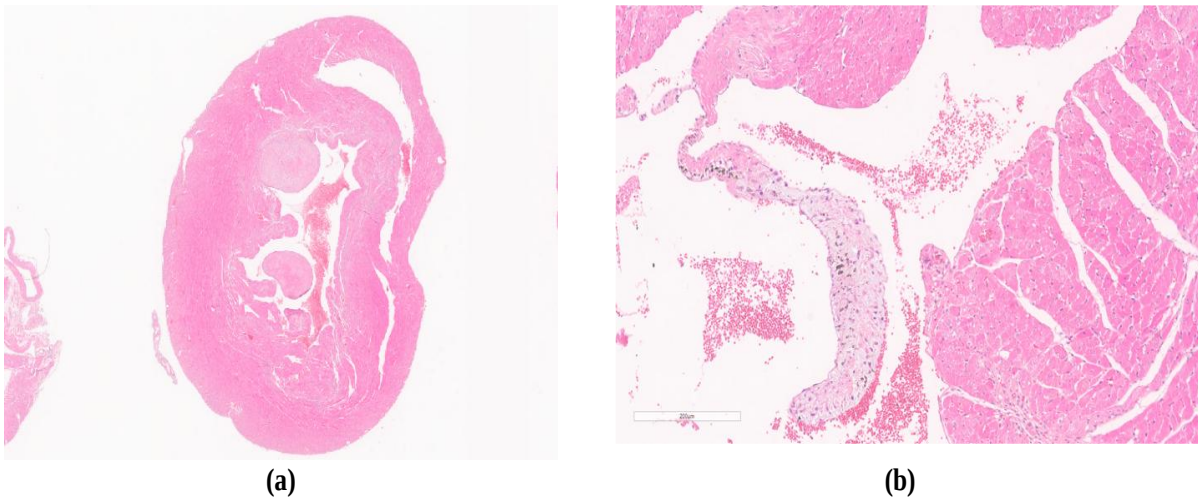

Figure 5.
Cross-section through the heart (G4, D25), a: fibrous tissue disposed in the inner third of the myocardium, b: detail for cardiomyocytes with degenerative changes separated by fibrosis

In contrast to G2-4, where the cardiac injury was confirmed through histological analysis, the control group (G5) showed no morphological changes in the heart wall (endocardium, myocardium, epicardium) or the heart valves (Figure 6a, b).

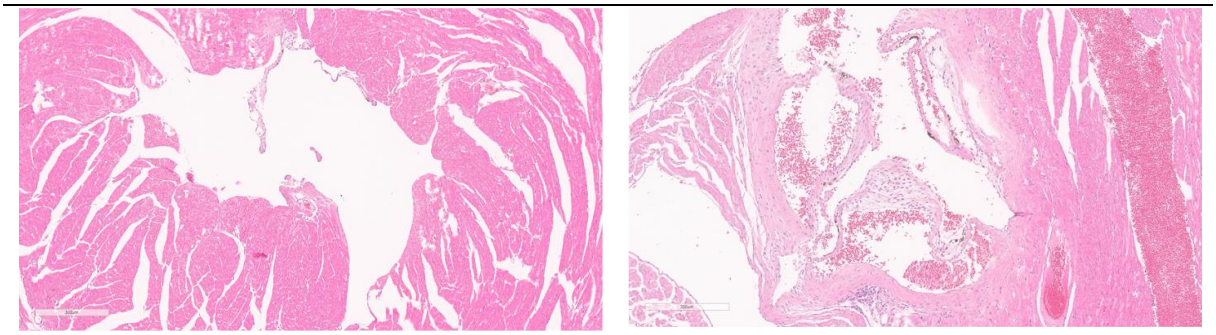

Figure 6.
The control group (G5) is a cross-section through the heart (a) and heart valves (b) with normal histology

The histological analysis of the ISO groups (G1 - ISO 160, G2 - ISO 80, G3 - ISO 40, G4 - ISO 20) compared to the control group (G5) provides strong evidence of morphological myocardial lesions resulting from ISO administration. Key characteristics of these lesions included chronic inflammation associated with acute-type cellular elements, with the most pronounced fibrosis observed in G4 (ISO 20). Additionally, cardiomyocyte damage in the ISO groups was focal, indicative of degenerative changes without clear signs of necrosis.

The review of the literature shows that the administration of ISO to mice, either through traditional methods (i.p. or s.c. injections) or by using an osmotic pump (that provides continuous administration), has been associated with the development of ventricular hypertrophy, dilation and dysfunction [2, 4, 12, 22]. At the same time, it was observed that ISO causes apoptosis of cardiomyocytes, which affects the contractility of the ventricle [2, 22]. Additionally, there were notable variations in left ventricular mass, hypertrophy, the degree of ventricular dilatation, and LVEF values among different mouse strains or species, indicating that the ISO dosage and the obtained cardiac outcomes need to be correlated with the genetic makeup of the mice [4, 12].

By integrating the findings from the echocardiographic assessments with the histopathological results, we conclude that the most optimal model for inducing HF in our experimental setup is the administration of a lower ISO dose (20 mg/kg) over a longer duration (25 days).

Conclusions

Isoproterenol (ISO) is a widely utilized pharmacological agent to induce cardiac dysfunction and remodelling based on persistent beta-adrenergic stimulation. ISO stimulates β1-adrenergic receptors, producing positive inotropic and chronotropic effects and promoting myocardial fibrosis. A study was conducted using different ISO doses administered over various durations to establish an optimal murine model of ISO-induced HF. Echocardiographic and histopathological analyses confirmed a progressive decline in cardiac function in all ISO-treated groups. Key echocardiographic parameters such as EF, FS, and LV mass (all with more robust measurements in PSAX view) demonstrated significant alterations. Histological evidence, such as cardiomyocyte degeneration and fibrous connective tissue within the myocardium, also reinforces this result. The administration of ISO at 20 mg/kg for 25 days (G4) emerged as the optimal protocol, balancing efficacy and feasibility with more uniform outcomes across the group. In this case, at the end of the experiment, a significant reduction in LV systolic function was observed, with an EF of 41.53%, while the values recorded for the control group (G5) were 60.66%. Moreover, G4 exhibited no mortality compared to higher-dose groups (40 - 50%), and the histopathological analyses revealed the most pronounced fibrosis, making it a reliable and reproducible model for HF induction.

Conflict of interest

The authors declare no conflict of interest.

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