Supplementary material

Procedural details of the *in vivo* experimental research

Experimental Animal Housing

Rats were housed in a dedicated animal maintenance room at the Animal Research Facility under controlled environmental conditions, including a temperature of 24–26°C, relative humidity of 65–75%, continuous air circulation with an exhaust fan, and a 12-hour light/12-hour dark artificial lighting cycle. Animals were kept in communal polypropylene cages (50 cm × 40 cm × 20 cm) fitted with stainless steel grid covers and wood shavings as bedding material. Each cage housed two rats. Cages were cleaned and bedding was replaced every 3 days. Body weight was measured upon arrival and subsequently monitored weekly.

Normal Diet

A normal diet was provided to all groups during the acclimatization period. Standard chow and drinking water were available *ad libitum*, with an estimated dietary intake of 20–30 g per rat per day. Food was placed on the wire cage cover, while drinking water was supplied in bottles attached to the cage cover. Food and fluid intake were recorded and measured daily. Food intake was calculated as the difference between the amount of food provided on the previous day and the remaining food prior to replenishment each morning.

High-Fat High-Fructose (HFHF) Diet

Following the acclimatization period, a high-fat high-fructose (HFHF) diet was administered until the end of the study to induce diabetic cardiomyopathy. The high-fat diet consisted of pellets containing 458.32 kcal/100 g, composed of 32.48% fat, 10.70% protein, 51.05%

carbohydrates, 10.09% moisture, and 4.68% ash, obtained from Universitas Brawijaya, Malang, Indonesia.

The high-fructose component was administered as a 55% fructose solution at a dose of 1.5 mL via intragastric gavage twice daily. The use of the HFHF diet was intended to accelerate myocardial tissue injury, thereby enabling detailed investigation of the morphological, biochemical, and functional characteristics underlying the pathogenesis of cardiovascular abnormalities.

Blood Glucose Measurement

Blood glucose levels were measured using an enzymatic method after rats were fasted for 6–8 hours. Fasting blood glucose was assessed weekly throughout the study until its completion, including prior to initiation of the high-fat high-fructose (HFHF) diet to obtain baseline glucose levels, prior to 5-MTP treatment, and at the end of the treatment period before euthanasia. Blood samples were obtained from the lateral tail vein, and blood glucose levels were measured using a glucometer.

Measurement of NT-proBNP Levels

Serum NT-proBNP levels were measured using a commercially available ELISA kit (Elabscience®, E-EL-R3023, Wuhan, China) according to the manufacturer's instructions. Briefly, serum samples and ELISA reagents were removed from refrigerated storage and equilibrated to room temperature. Samples were centrifuged (spin-down) at 8,000 rpm for 2 minutes, after which standard solutions and test samples were prepared.

A total of 100 μL of standards or samples was added to each well and incubated for 90 minutes at 37°C. The liquid was then discarded, followed immediately by addition of 100 μL biotinylated detection antibody solution to each well, with incubation for 60 minutes at 37°C. After incubation, the plate was aspirated and washed three times. Subsequently, 100 μL of streptavidin-HRP working solution was added to each well and incubated for 30 minutes at 37°C. The plate was then aspirated and washed five times.

Next, 90 μL of TMB substrate solution was added to each well and incubated for 15 minutes at 37°C. The reaction was terminated by adding 50 μL stop solution. Absorbance was measured immediately at 450 nm, and NT-proBNP concentrations were calculated according to the standard curve.

Supplementary Table 1 Fasting blood glucose levels from the pilot model-characterization study of the HFHF/STZ-induced early-stage diabetic cardiomyopathy model.

Group (n=4/group)	FBG post HFHF and STZ		
	Mean ± SD	P-value	P-value
Control	87,3 ± 8,1		0,035*[c]
HFHF + STZ25 mg/kgBW	240,0 ± 67,7	0,008*	1,000[a]
HFHF + STZ 40 mg/kgBW	208,3 ± 64,8		0,010*[b]

[a] HFHF + STZ 25 mg/kgBW vs HFHF + STZ 40 mg/kgBW.

[b] HFHF + STZ 40 mg/kgBW vs Control.

[c] HFHF + STZ 25 mg/kgBW vs Control.

Supplementary Table 2 Serum NT-proBNP levels from the pilot model-characterization study of the HFHF/STZ-induced early-stage diabetic cardiomyopathy model.

Group (n=4/group)	NT-proBNP Levels		
	Mean ± SD (n = 12)	P-value	P-value
Control	624,3 ± 126,4	0,001*	0,018*[a]
HFHF + STZ25 mg/kgBW	1121,7 ± 222,5		0,001*[b]
HFHF + STZ 40 mg/kgBW	1367,6 ± 225,0		0,332[c]

[a] HFHF + STZ 25 mg/kgBW vs Control.

[b] HFHF + STZ 40 mg/kgBW vs Control.

[c] HFHF + STZ 25 mg/kgBW vs HFHF + STZ 40 mg/kgBW.

Supplementary Table 3. Baseline characteristics of the experimental groups before initiation of 5-MTP treatment.

8- day treatment duration group

Parameter	DCM	DCM + 5-MTP 25 mg	DCM + 5-MTP 50 mg	DCM + 5-MTP 100 mg	P Value
Initial body length	19.3 ± 0.9	19.9 ± 1.3	20.3 ± 2.2	19.3 ± 1.0	0.703
Body length after STZ induction	20.8 ± 1.4	20.9 ± 1.3	21.9 ± 0.9	20.4 ± 0.5	0.311
Initial body weight	213 (208–258)	239 (214–270)	225 (214–272)	218 (210–234)	0.434
Body weight after STZ induction	308.5 ± 98.0	299.5 ± 59.8	256.5 ± 45.4	266.0 ± 45.3	0.633
Initial fasting blood glucose level	99.3 ± 9.3	100.0 ± 10.7	98.0 ± 16.6	88.3 ± 10.7	0.511
Fasting blood glucose level after STZ induction	249.5 ± 101.9	213.5 ± 53.0	201.8 ± 3.6	221.3 ± 93.2	0.824

Data are presented as mean ± SD or median (interquartile range), as appropriate. P values represent overall comparisons among the four treatment groups using one-way ANOVA or Kruskal–Wallis test according to data distribution.

16- day treatment duration group

Parameter	DCM	DCM + 5-MTP 25 mg	DCM + 5-MTP 50 mg	DCM + 5-MTP 100 mg	P Value
Initial body length	19.8 ± 1.9	19.1 ± 0.6	19.3 ± 1.0	19.8 ± 1.0	0.832
Body length after STZ induction	21.8 (20.5–22.0)	20.0 (20.0–21.0)	20.8 (20.0–21.5)	20.5 (20.0–22.0)	0.156
Initial body weight	229.5 ± 25.1	223.0 ± 17.5	219.5 ± 6.4	215.5 ± 4.4	0.648
Body weight after STZ induction	232.5 ± 17.9	262.0 ± 46.7	277.0 ± 55.2	227.0 ± 22.8	0.264
Initial fasting blood glucose level	100.5 ± 12.0	113.0 ± 8.6	86.3 ± 26.1	89.3 ± 14.3	0.148
Fasting blood glucose level after STZ induction	244.8 ± 141.2	213.5 ± 39.0	274.5 ± 54.7	204.0 ± 49.4	0.621

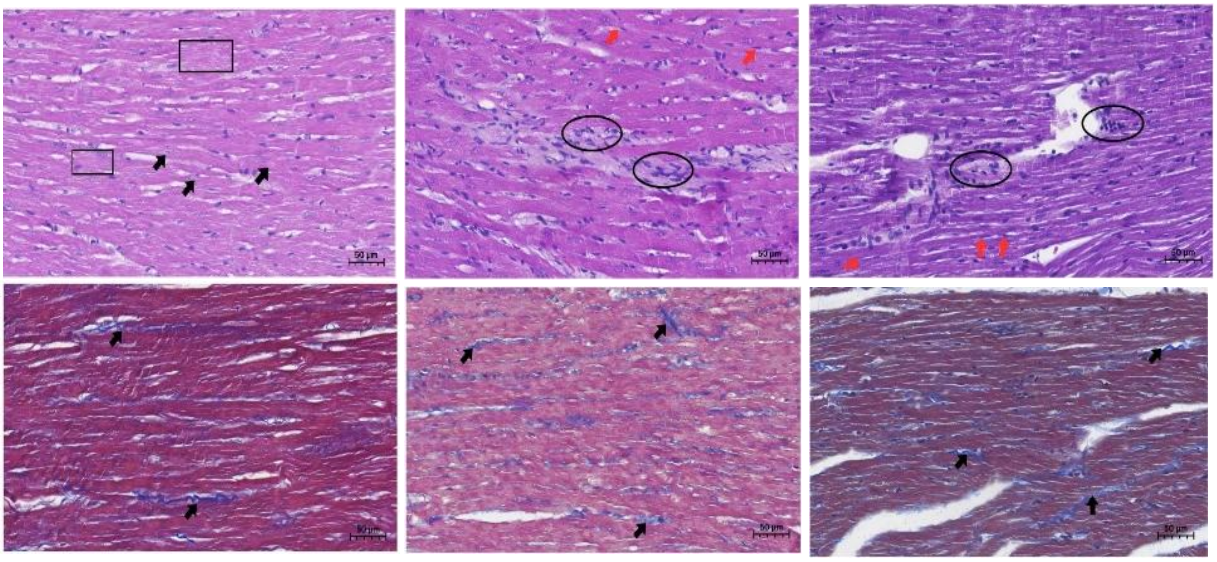
Data are presented as mean ± SD or median (interquartile range), as appropriate. P values represent overall comparisons among the four treatment groups using one-way ANOVA or Kruskal–Wallis test according to data distribution.

32- day treatment duration group

Parameter	DCM	DCM + 5-MTP 25 mg	DCM + 5-MTP 50 mg	DCM + 5-MTP 100 mg	P Value
Initial body length	19.3 ± 1.5	19.9 ± 0.9	19.0 ± 0.8	19.3 ± 1.7	0.797
Body length after STZ induction	21.0 (21.0–22.0)	20.8 (20.0–22.0)	20.5 (19.0–21.0)	21.0 (20.0–22.0)	0.417
Initial body weight	233.5 ± 22.1	226.5 ± 28.9	233.0 ± 6.2	214.0 ± 12.4	0.483
Body weight after STZ induction	209.5 ± 38.9	266.5 ± 32.6	275.5 ± 20.5	239.5 ± 14.2	0.026
Initial fasting blood glucose level	94.3 ± 22.3	93.3 ± 14.8	95.5 ± 13.2	91.3 ± 11.8	0.984
Fasting blood glucose level after STZ induction	289.8 ± 106.1	230.3 ± 49.8	284.5 ± 91.4	209.8 ± 89.1	0.498

Data are presented as mean ± SD or median (interquartile range), as appropriate. P values represent overall comparisons among the four treatment groups using one-way ANOVA or Kruskal–Wallis test according to data distribution.

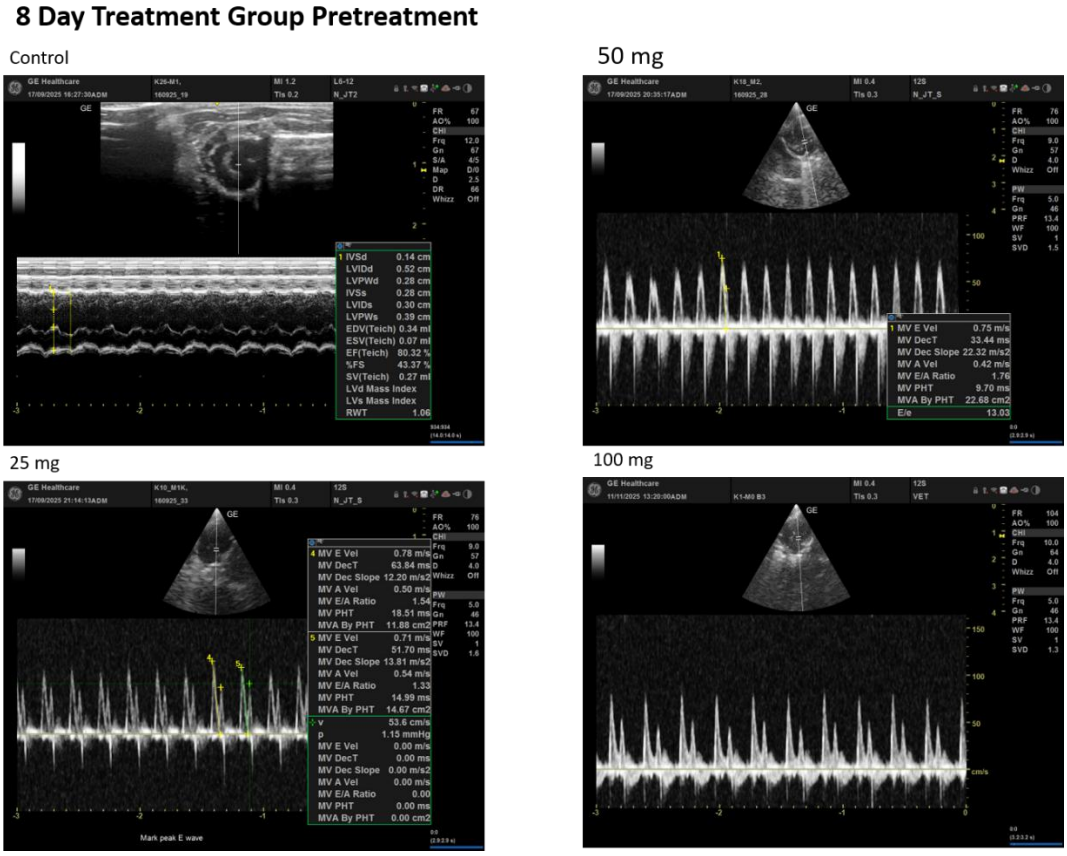
Control **HFHF+STZ 25 mg/kgBW** **HFHF+STZ 40**



Supplementary Figure 1 Representative myocardial histopathological findings from the pilot model-characterization study of the HFHF/STZ-induced early-stage diabetic cardiomyopathy model. Representative myocardial sections stained with Hematoxylin–Eosin (HE, upper panels) and Masson's Trichrome (MT, lower panels). Representative HE-stained myocardial sections from the control group and STZ-treated groups are shown. The control group demonstrates preserved myocardial architecture with uniformly shaped cardiomyocytes, eosinophilic cytoplasm, visible cross-striations (rectangles), and centrally located oval nuclei (black arrows). In the STZ-treated groups, myocardial architecture shows progressive alterations, including irregular arrangement of cardiomyocytes, variation in cell size and shape, and inflammatory cell infiltration (ovals). Increased numbers of fibroblasts are also observed (red arrows), accompanied by reduced cytoplasmic striations and nuclear changes. (Hematoxylin–Eosin ×200). Representative Masson's Trichrome–stained myocardial sections demonstrate minimal interstitial collagen deposition in the control group, whereas the STZ-treated groups show increased blue-stained collagen within the interstitial and perivascular areas. The extent of collagen deposition appears greater in the STZ 40 mg/kg group than in

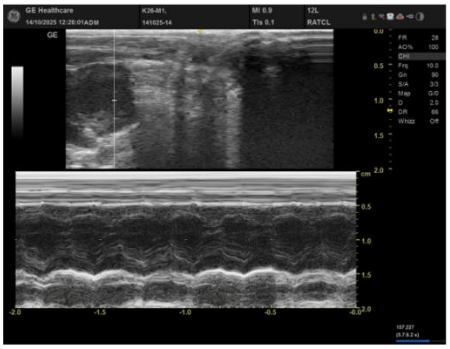
the STZ 25 mg/kg group, indicating a graded pattern of fibrotic remodeling across STZ doses. (Masson's Trichrome ×200).

Supplementary material Echocardiography parameters pre and post MTP in each treatment group



8 Day Treatment Group Posttreatment

Control



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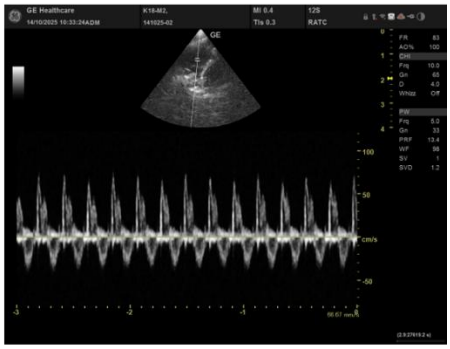

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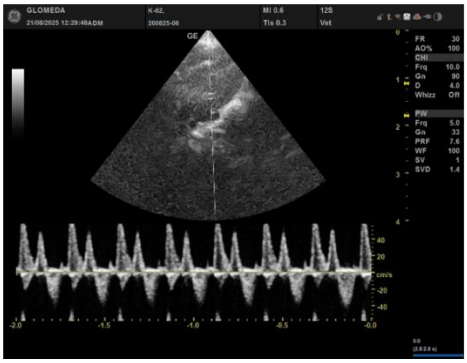

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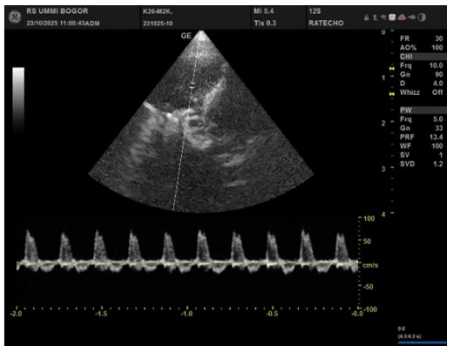
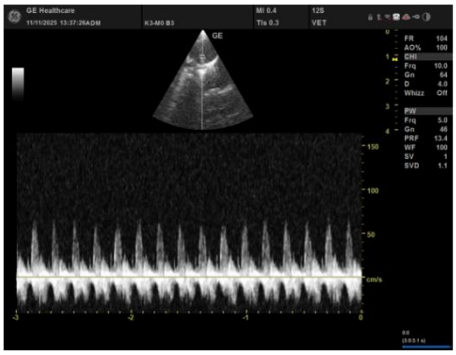


16 Day Treatment Group Pretreatment

Control



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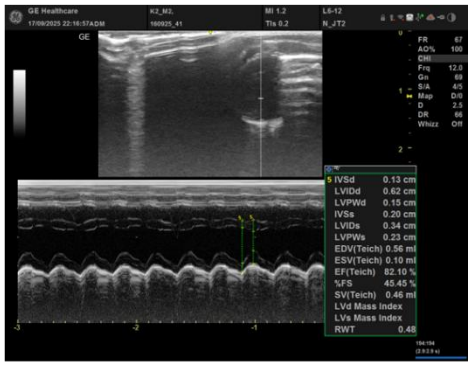

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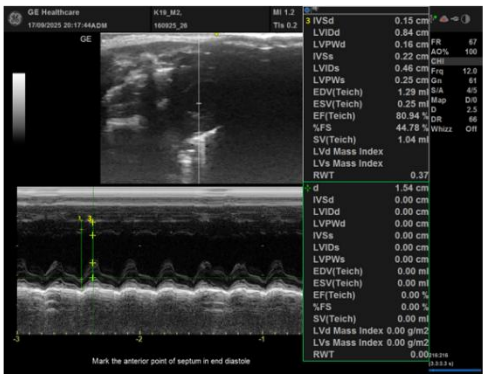

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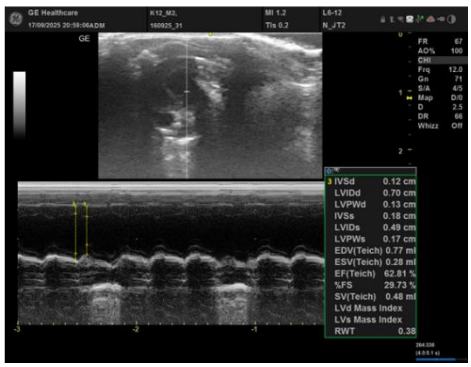
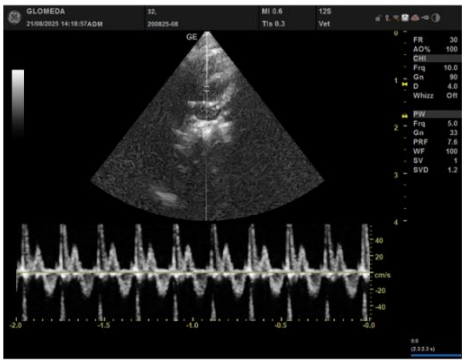


16 Day Treatment Group Posttreaetment

Control



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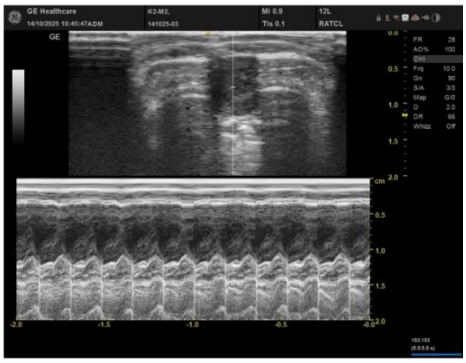

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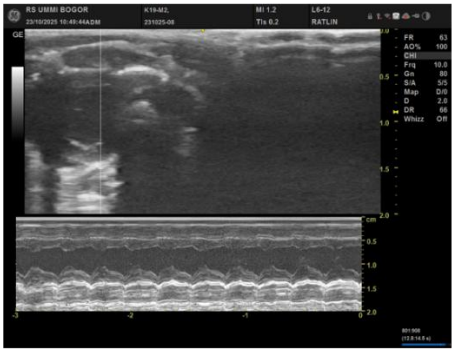

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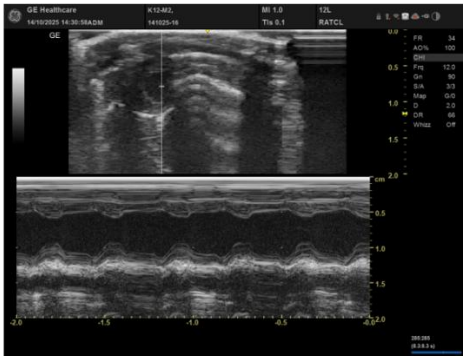
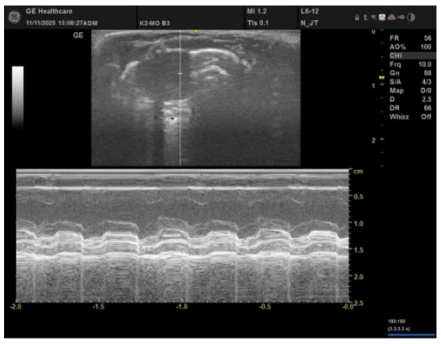


32 Day Treatment Group Pretreatment

Control



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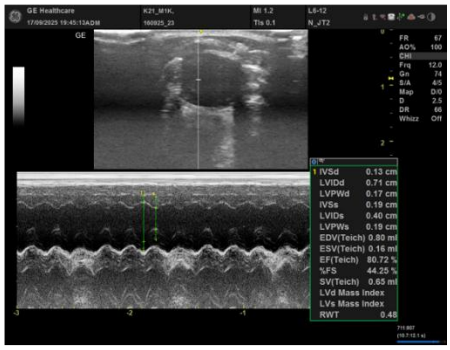

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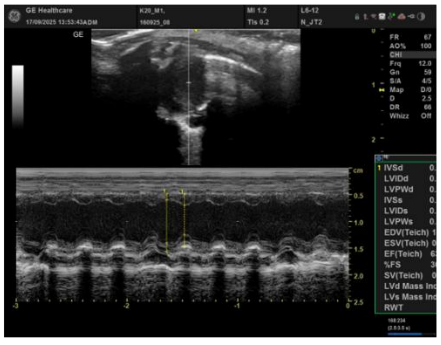

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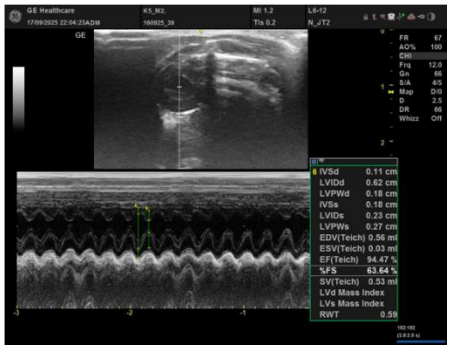
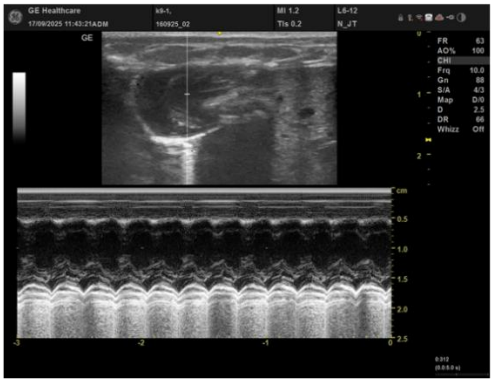


32 Day Treatment Group Posttreatment

Control



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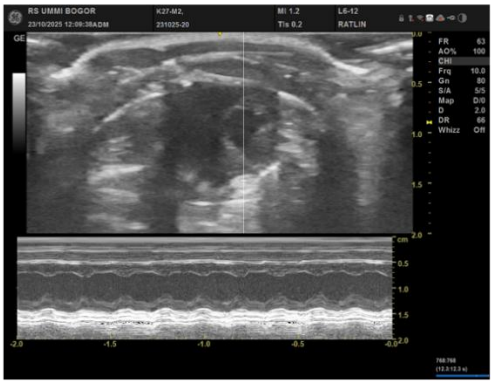

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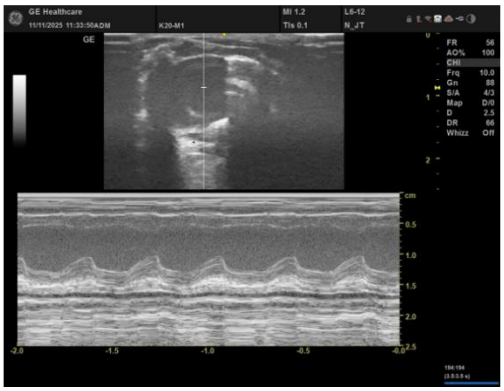

100 mg