Supplementary information

Lithium treatment induces cardiac dysfunction in mice

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Ethical Considerations

The study was approved by the Local Ethical Panel and Italian Ministry of Health (Prot. n° 839/2021-PR) and conforms to principles of laboratory animal care demanded by European Directive and Italian laws. The study aimed to investigate the potential adverse effects of administering lithium salts to mice on the electrical function of the heart, cardiotoxicity and liver and kidney alterations. This study was multi-organ, and to fully understand its impact, it could only be conducted in vivo, using the entire morpho-functional model. Currently, therefore, no replacement for the animal model is available. In our experimental design, we opted for non-invasive methods under general anaesthesia, commonly used in human and veterinary clinics, and administered preparations orally to reduce animal manipulation. We monitored the health conditions of the animals daily and chose a species-specific euthanasia method, i.e., anaesthetic overdose, permitted by National and European laws. All procedures were carried out by adequately trained personnel.

Table S1. HF-US **cardiac parameters at T0 and T1**

	C-group		Li-group	
	T_0	T_1	T_0	T_1
Vol_s (µl)	44.3 [13.2]	34.8 [23.5]	43.2 [10.3]	36.9 [26.0]
Vol_d (µl)	87.4 [12.8]	77.3 [32.7]	83.3 [27.0]	76.7 [38.8]
SV (µl)	37.5 [9.9]	42,4 [10.1]	38.4 [18.3]	41.6 [14.9]
EF%	49.7 [8.3]	51 [4.9]	53.0 [6.2]	40.0 [7.4] **†
FS%	14.7 [5.0]	13.0 [5.5]	17.3 [5.7]	10.4 [3.9] * †
LVmass (mg)	119.3 [24.6]	113.1 [26.0]	128.8 [46.4]	124.0 [51.7]
CO (ml/min)	14.2 [7.4]	20.0 [5.7]	18.0 [9.7]	17.3 [4.7]
E/A (a.u.)	1.3 [0.5]	1.5 [0.6]	1.6 [0.7]	1.3 [0.3]
IVRT (ms)	15.2 [5.0]	15.0 [72]	15.0 [4.2]	18.1 [6.4]
IVCT (ms)	14.3 [2.1]	14.6 [5.3]	11.1 [3.8]	14.3 [3.7]
A_{et} (ms)	56.2 [10.8]	55.6 [18.2]	51.7 [9.5]	52.9 [10.4]

Values are median [IQR], n=8 for each group. Vol_s: systolic volume; Vol_d: diastolic volume; SV: stroke volume; EF%: ejection fraction; FS%: fractional shortening; LVmass: left ventricular mass; CO: cardiac output; E/A: E/A ratio. IVRT: isovolumic relaxation time; IVCT: isovolumic contraction time; A_{et}: aortic ejection time.

*p<0.05 and **p<0.005 vs C–group at the correspondent time point; †p<0.05 for paired comparison vs T_0 within each group.

Table S2. Electrocardiographic characteristics of the two groups at T_0 and T_1

	C-group		Li-group	
	T_0	T_1	T_0	T_1
HR (bpm)	528 [87]	513 [101]	527 [68]	515 [100]
P duration (ms)	11.1 [2.0]	10.8 [1.1]	10.8 [2.5]	9.9 [1.4]
PR interval (ms)	36.6 [2.7]	37.0 [8.7]	37.3 [3.8]	41.2 [7.0]
QRS duration (ms)	10.0 [1.0]	9.6 [1.9]	9.9 [1.0]	10.2 [1.3]
QTc interval (ms)	46.4 [3.5]	45.2 [2.0]	46.6 [1.7]	51.4 [2.9] **§
JTc interval (ms)	36.4 [3.9]	37.0 [3.6]	36.4 [1.8]	40.7 [2.9] *§

Values are median [IQR], n=8 for each group. HR: heart rate; bpm: beat per minute; P: wave of atrial depolarisation; PR: interval from the beginning of the P wave to the beginning of the QRS complex; QRS: complex of ventricle depolarisation; QTc: QT interval (time of ventricle depolarisation and repolarisation) corrected for HR; JTc: JT interval (time of ventricle repolarisation) corrected for HR. *p<0.01 and **p<0.001 vs C–group at the correspondent time point; §p<0.05 for paired comparison vs T_0 within each group.