

Supplementary material to:

ROSELLE ATTENUATES CARDIAC HYPERTROPHY AFTER MYOCARDIAL INFARCTION *IN VIVO* AND *IN VITRO*

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Supplementary Table 1: Primer sequences used in gene expression study

Gene	Forward (5'-3')	Reverse (5'-3')
18S	TTCGAGGCCCTGTAATTGGA	GCAGCAACTTTAATATAGGCTATTGG
ANP	GGAAGTCAACCCGTCTCAGA	TGGGCTCCAATCCTGTCAAT
BNP	ACAAGAGAGAGCAGGACACC	TCTGGAGACTGGCTAGGACT
Col 1	TGCTGCCTTTTCTGTTCTT	AAGGTGCTGGGTAGGGAAGT
Col 3	GTCCACGAGGTGACAAAGGT	CATCTTTTCCAGGAGGTCCA

Supplementary Table 2: Raw data to body weight gained, heart weight, heart weight/tibia length, systolic blood pressure (SBP) and heart rate (HR) of the rats (Table 1, main document); n=5-6; outliers were excluded based on GraphPad QuickCalcs.

Body weight gained (g)			
	Control	MI	MI+R
n1	68.00	27.8	58.0
n2	65.0	64.4	50.0
n3	41.0	74.0	56.0
n4	22.0	66.4	50.0
n5	48.0	39.8	58.0
n6	56.0	54.5	49.0
Heart weight (g)			
	Control	MI	MI+R
n1	1.01	1.14	1.24
n2	1.39	1.44	0.81
n3	0.94	1.13	1.11
n4	0.88	1.33	1.13
n5	0.98	1.38	1.12
n6	1.02	1.25	1.09
Heart weight/Tibia length (g/cm)			
	Control	MI	MI+R
n1	0.224	0.265	0.282
n2	0.331	0.360	0.189
n3	0.219	0.263	0.264
n4	0.215	0.309	0.282
n5	0.239	0.337	0.273
n6	0.243	0.298	0.273
Systolic blood pressure (mmHg)			
	Control	MI	MI+R
n1	131.0	136.0	144.5
n2	132.0	144.7	139.5
n3	117.0	153.7	131.4
n4	132.7	166.0	116.2
n5	129.5	173.0	150.8
n6	134.7	165.0	121.0
Heart rate (bpm)			
	Control	MI	MI+R
n1	315	313	328
n2	307	341	325
n3	311	371	300
n4	301	348	295
n5	291	367	326
n6	300		338

Supplementary Table 3: Raw data to left ventricular developed pressure (LVDP), left ventricular (LV) maximum and minimum rate of pressure changes (LVdP/dt_{max} and LVdP/dt_{min}), time constant of isovolumic relaxation (Tau) and coronary flow. (Figure 2, main document); n=4-6; outliers were excluded based on GraphPad QuickCalcs.

LVDP (mmHg)			
	Control	MI	MI+R
n1	92.69	27.80	55.95
n2	81.53	26.17	62.80
n3	113.06	29.13	33.34
n4	121.18	45.82	38.90
n5	118.0	21.02	
n6	93.04	20.98	
LVdP/dt_{max} (mmHg/s)			
	Control	MI	MI+R
n1	1770.08	859.89	1290.89
n2	1293.35	772.77	1175.01
n3	1854.45	752.84	1896.74
n4	1848.96	891.53	1259.31
n5	1685.26	634.73	
n6	1815.28	828.82	
LVdP/dt_{min} (mmHg/s)			
	Control	MI	MI+R
n1	919.75	771.42	754.24
n2	766.36	362.55	503.43
n3	807.26	359.71	672.66
n4	1059.20	534.85	815.36
n5	1111.32	242.37	
n6	1027.07	243.48	
Tau (m/s)			
	Control	MI	MI+R
n1	0.31	0.46	0.39
n2	0.16	0.67	0.31
n3	0.14	0.57	0.33
n4	0.27	0.56	0.42
n5	0.15	0.58	
n6	0.29	0.46	
Coronary flow (ml/min)			
	Control	MI	MI+R
n1	15.51	0.80	9.39
n2	7.90	3.00	11.00
n3	13.03	1.91	10.48
n4	13.01	3.61	11.72
n5	11.15	7.93	
n6	11.49	6.72	

Supplementary Table 4: Raw data to mRNA expression of subunit of NADPH oxidase (NOX2), 8-Isoprostane level, superoxide dismutase (SOD) enzyme activity, glutathione level of the rats (Figure 3, main document); n=4-6; outliers were excluded based on GraphPad QuickCalcs.

NOX2 mRNA expression (fold, control)			
	Control	MI	MI+R
Mean±SEM (pooled sample)	1.00±0.09	2.22±0.34	0.52±0.21
8-Isoprostane level (pg/ml/mg protein)			
	Control	MI	MI+R
n1	0.41	0.29	0.39
n2	0.43	0.33	0.40
n3	0.40	0.32	0.36
n4	0.42	0.35	0.39
n5	0.43	0.35	0.41
n6	0.43	0.34	0.42
SOD enzyme activity (U/mg protein)			
	Control	MI	MI+R
n1	0.033	0.027	0.027
n2	0.035	0.026	0.032
n3	0.034	0.026	0.028
n4	0.034	0.027	0.031
n5	0.035	0.029	0.034
n6	0.036	0.030	0.034
GSH level (mmol/mg protein)			
	Control	MI	MI+R
n1	0.31	0.46	0.39
n2	0.16	0.67	0.31
n3	0.14	0.57	0.33
n4	0.27	0.56	0.42
n5	0.15	0.58	
n6	0.29	0.46	

Supplementary Table 5: Raw data to cardiomyocyte cross-sectional area, collagen %, mRNA expression of atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), collagen 1, and collagen 3 of the rats (Figure 4, main document); n=4-6; outliers were excluded based on GraphPad QuickCalcs.

Cardiomyocyte cross-sectional Area (μm^2)			
	Control	MI	MI+R
n1	12674.96	13655.14	11588.70
n2	12356.42	14711.00	11817.88
n3	10989.00	17331.58	11359.94
n4	11055.54	12894.80	12248.26
n5	10760.22	14077.18	12693.14
n6	13378.10	17571.72	14246.36
Collagen (%)			
	Control	MI	MI+R
n1	0.11	12.59	8.29
n2	0.13	12.23	6.93
n3	0.17	14.87	8.37
n4	0.11	16.00	10.30
n5	0.13	14.81	10.01
n6	0.19	13.43	7.03
ANP (fold, control)			
	Control	MI	MI+R
Mean\pmSEM (pooled sample)	1.00 \pm 0.54	6.20 \pm 4.80	4.41 \pm 1.97
BNP (fold, control)			
	Control	MI	MI+R
Mean\pmSEM (pooled sample)	1.00 \pm 0.53	2.20 \pm 0.44	0.56 \pm 0.21
Collagen 1 (fold, control)			
	Control	MI	MI+R
Mean\pmSEM (pooled sample)	1.00 \pm 0.11	4.71 \pm 0.94	3.51 \pm 1.43
Collagen 3 (fold, control)			
	Control	MI	MI+R
Mean\pmSEM (pooled sample)	1.00 \pm 0.65	8.73 \pm 1.73	1.21 \pm 0.64

Supplementary Table 6: Raw data to cell viability of H9c2 cell line against roselle extract at different concentration and cellular area of each experimental group in pre-, simultaneous, and post-treatment experiments.(Figure 5, main document); n=3 independent experiments; outliers were excluded based on GraphPad QuickCalcs.

Cell viability (%)									
Roselle (mg/ml)	15	10	7.5	5	2.5	1.25	0.625	0.3125	0
n1		11.9		78.6	98.4	98.4	106.8	102.9	106.4
n2	14.9	7.3	45.5	98.5	136.3	136.3	143.3	129.2	118.6
n3	12.3	5.4	39.2	82.6	118.2	118.2	134.9	124.0	129.8

Cellular area pre-treatment (mm ²)						
	Control	AngII	10 µg/mL R	AngII + 10 µg/mL R	AngII + 5 µg/mL R	AngII + 1 µg/mL R
n1	0.003226	0.005221	0.003346	0.003093	0.003006	0.002915
n2	0.003294	0.005112	0.004128	0.004464	0.004293	0.003287
n3	0.004272	0.005742	0.003363	0.004479	0.003776	0.004243

Cellular area simultaneous-treatment (mm ²)						
	Control	AngII	10 µg/mL R	AngII + 10 µg/mL R	AngII + 5 µg/mL R	AngII + 1 µg/mL R
n1	0.004763	0.007046	0.004072	0.005259	0.003896	0.003629
n2	0.003877	0.005358	0.003201	0.003674	0.004084	0.003936
n3	0.004322	0.005165	0.00368	0.003022	0.003878	0.003802

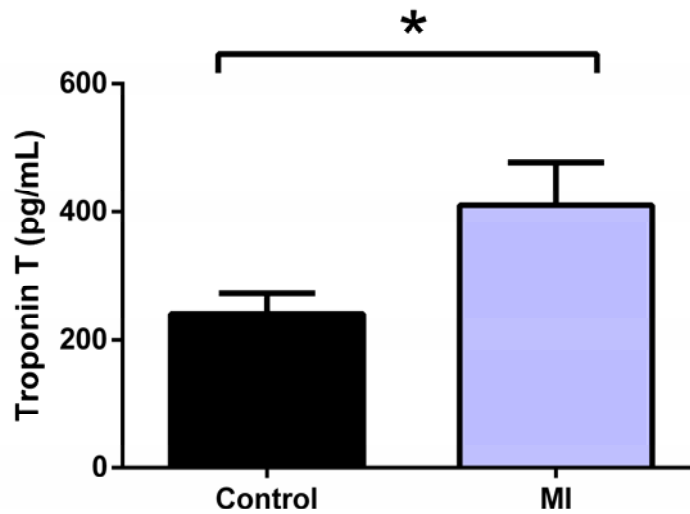
Cellular area post-treatment (mm ²)						
	Control	AngII	10 µg/mL R	AngII + 10 µg/mL R	AngII + 5 µg/mL R	AngII + 1 µg/mL R
n1	0.003705	0.004282	0.003772	0.003602	0.00375	0.003673
n2	0.003106	0.005045	0.003136	0.003324	0.003678	0.003566
n3	0.004212	0.005438	0.003023	0.002887	0.00261	0.003084

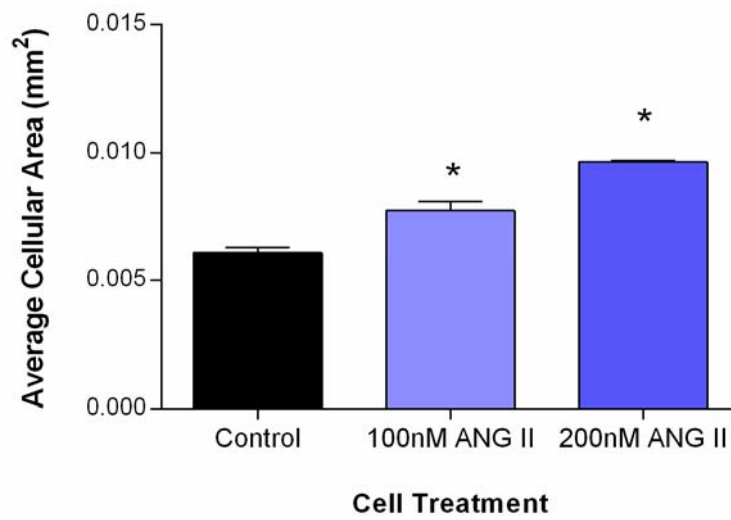
Supplementary Table 7: Raw data to plasma troponin T of the rats. (Supplementary Figure 1, supplementary document); n=6

Troponin T (pg/ml)		
	Control	MI
n1	209.38	419.22
n2	286.09	371.41
n3	234.38	410.94
n4	223.91	503.13
n5	273.28	387.50
n6	218.13	346.09

Supplementary Table 8: Raw data to cellular area of H9c2 cell line with 24H incubation of angiotensin II. (Supplementary Figure 2, supplementary document); n=2 independent experiments; outliers were excluded based on GraphPad QuickCalcs.

Cellular area (mm ²)			
	Control	100 nM Ang II	200 nM Ang II
n1	0.0059	0.00740	0.00960
n2	0.0063	0.00740	0.00970

**Supplementary Figure 1:** Plasma level of troponin T for validation of myocardial infarction. Values are presented as mean \pm SEM for n=6 per group. ^ap < 0.05 vs. Control



Supplementary Figure 2: Impact of ANG II on cardiomyocyte size after 24 h incubation. Values are presented as mean \pm SEM for n=3 independent experiments. ^ap < 0.05 vs. Control