

Supplementary Information

Preventive effect of Agnucastoside C against isoproterenol-induced myocardial injury

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IR, UV and NMR data of ACC

UV (MeOH) $\lambda_{\max}$ nm (log e): 215 (3.40);

IR: $V_{\max}$ (cm⁻¹) 3428, 2908, 1690, 1630, 1510, 1431, 1372, 1228, 1171, 915, 897, 831

HR- ESI-MS: m/z 707 [M+Na]⁺, C₃₄H₃₆O₁₅.

¹H NMR data. (400 MHz, DMSO-*d*₆, δ ppm): 5.17 (1H d, J=5.5 Hz, H-1), 7.28 (1H, s, H-3), 3.15 (1H, d, J=8.0 Hz, H-5), 1.21 (1H, m), 2.02 (1H, m, H-6), 4.90 (1H, m, H-7) 2.49 (1H, m, H-9), 1.06 (3H, d, J=7.0 Hz, H-10), 4.68 (d, J=8.0 Hz, H-1'); 3.31 (1H, m, H-2'); 3.40 (1H, d, J=7.0 Hz, H-3'); (3.55 (1H, m, H-5'); 4.40 (1H, d, J=, 6.0 Hz, H-6') ; 7.05 (1H, d, J=2.0 Hz, H-2'') ; 6.71 (1H, d, J=8.0 Hz, H-5'') ; 6.91 (1H, dd, J=8.0, 2.0 Hz, H-6'') ; 6.28 (1H, d, J=16.0 Hz, H-8'') ; 7.51 (1H, d, J=8.5 Hz, H-2''') ; 6.77 (1H, d, J=8.5 Hz, H-3''') ;) 6.77 (1H, d, J=8.5 Hz, H-5''') ; 6.29 (1H, d, J=16.0 Hz, H-8''')

¹³C-NMR data. (400 MHz, DMSO-*d*₆) 92.19 (C-1); 116.74 (C-3); 39.5 (C-6); 82.98 (C-7); 40.13 (C-8); 40.34 (C-9), 18.37 (C-10), 74.7 (C-2'); 77.1 (C-3'); 70.89 (C-4'); 77.58 (C-5'); 63.49 (C-6') ; 114.1 (C-2''); 116.7 (C-5'') 122.1 (C-6''); 146.2 (C-7'') 115.1 (C-8'') 166 (C-9''); 125 (C-1'''); 132 (C-2'''); 116.1 (C-3'''), 161 (C-4'''); 116.2 (C-5''') 146.2 (C-7'''); 115.2 (C-8'''); 168 (C-9''').

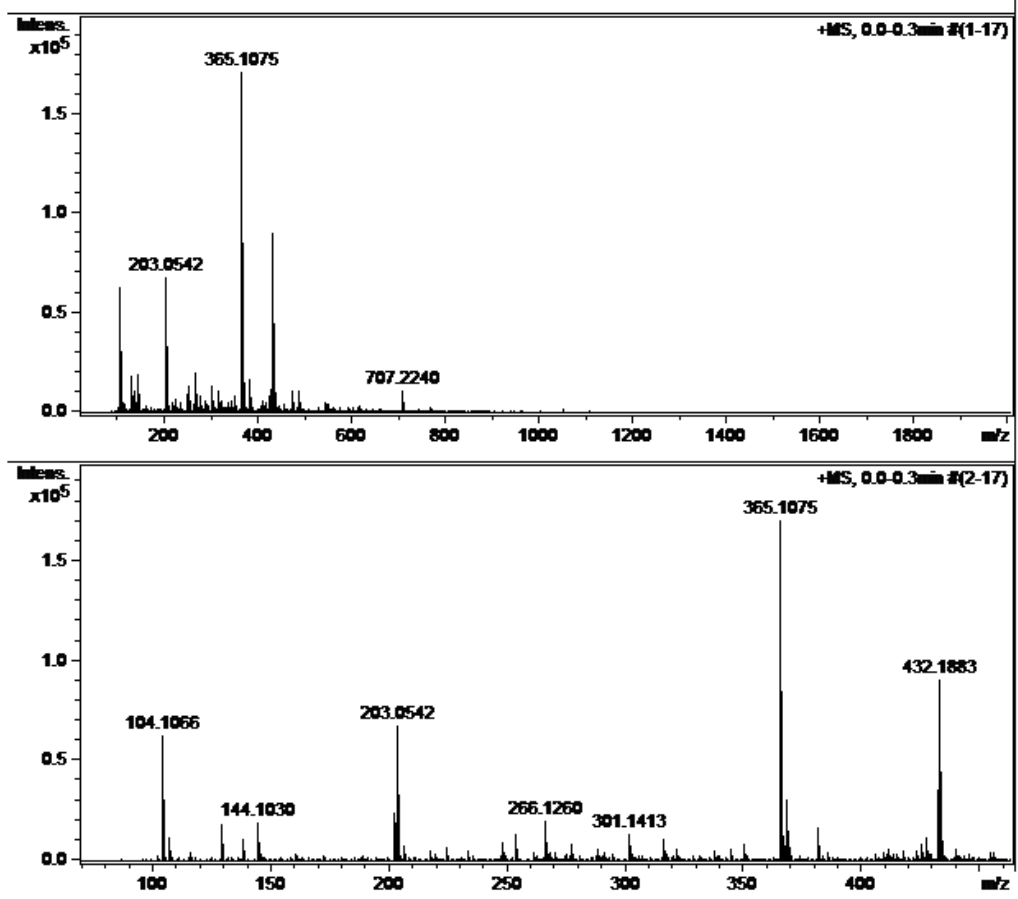


Figure S1



Figure S2(a)

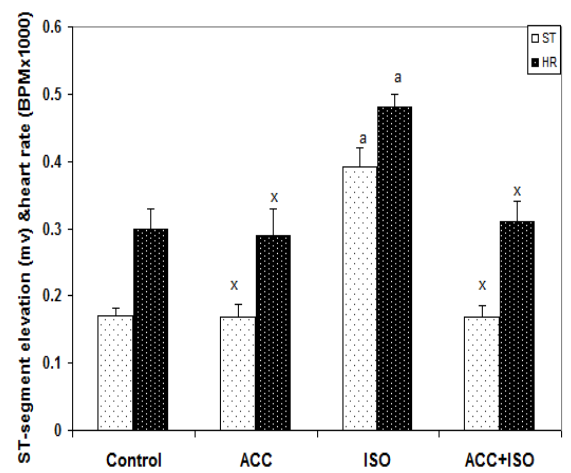


Figure S2 (b)

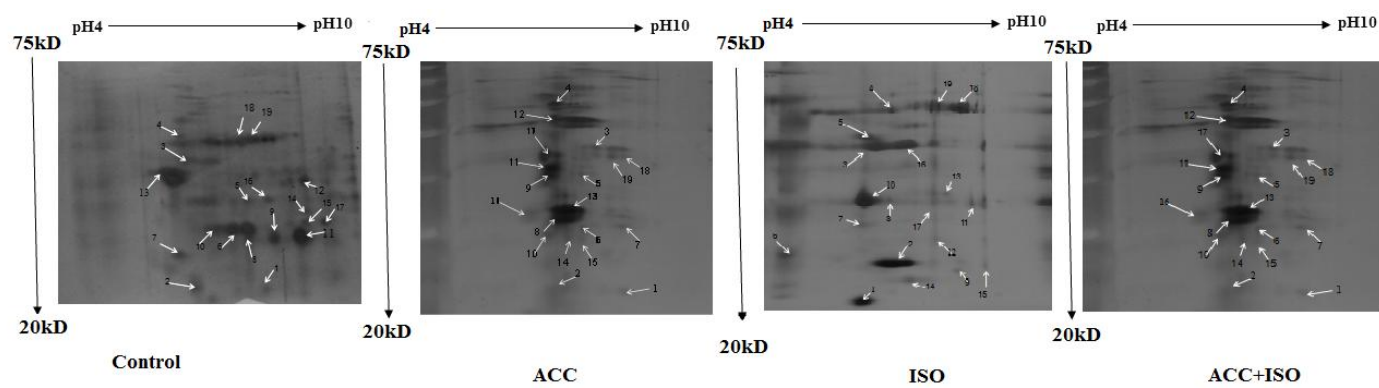


Figure S3

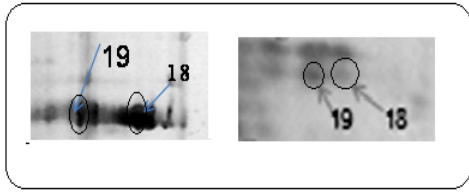


Figure S4 (a)

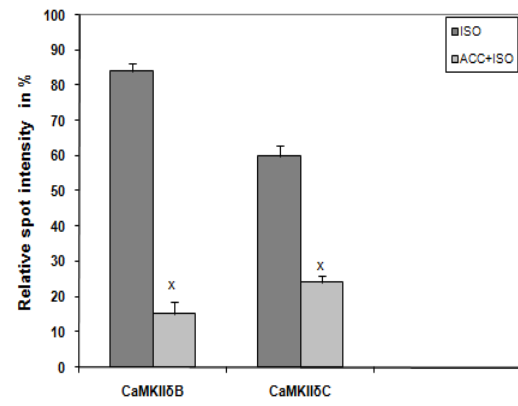


Figure S4(b)

Table S1. Changes in Body weight (B.wt.), Heart weight and Heart weight/Body weight (%) ratio after the administration of ACC.

	Final body weight (g)	Heart weight (g)	Heart weight/B. wt. (%)
Control	183.2 ±10.15	0.610 ±0.041	0.312 ±0.026
ISO	168.4 ^{ns} ±8.67	1.12 ^a ±0.088	0.694 ^x ±0.029
ACC	194.6 ^{ns} ±11.10	0.680 ^x ±0.061	0.401 ±0.024
ISO+ACC	187.56 ns ±9.93	0.650 ^x ±0.051	0.346 ^x ±0.030

Data are means ±S.E.M. (n=7). ^a*P*<0.001 as compared to the respective control values. ^x*P*<0.001 as compared to the respective values of the isoproterenol (ISO)-induced animals and ns–non significant (one-way ANOVA, followed by Student Newman-Keuls Post-hoc test).

Table S2 Scoring for histological alterations in myocardium in different groups of rats.

Group(s)	–	+	++	+++
Control	5	0	0	0
ACC	5	0	0	0
ISO	0	0	0	5
ACC+ISO	0	4	1	0

Photomicrographs were used to evaluate the cardiac necrosis in the heart tissue: (-) no changes; (+) mild changes; (++) moderate changes; (+++) marked changes.

Legends to figures.

Figure S1. Mass spectrum of isolated compound, agnucastoside C (positive ionization mode).

Figure S2 (a). Representative electrocardiogram (ECG) tracings of control, ACC, ISO and ACC+ ISO treated rats. **(b).** ECG showing changes in ST-segments and in heart beats of control and experimental animals. Values are expressed as mean $\pm$ S.E. (n = 7). (One-way ANOVA, followed by Student Newman-Keuls Post-hoc test). ^aP < 0.001 as compared to the respective control value. ^xP < 0.001 as compared to the respective value of the ISO- induced animals .

Figure S3. Representative 2D gel images, obtained from heart protein extracts in control, ACC, ISO and ACC pretreated ISO-induced rats.

Figure S4. (a). Selected regions of two-dimensional image showing protein spots 18 and 19, identified as CaMKIIdB and CaMKIIdC. These two spots show higher levels in ISO and lower levels in ACC+ ISO group. **(b).** The figure shows the statistical data of spots 18 and 19, displaying a significant decrease in spot intensity in ACC-pretreated ISO-induced rats compared with the ISO-alone group.