



Supplementary material

**Polyphenol-enriched Diet Prevents Coronary Endothelial Dysfunction
by Activating the Akt/eNOS Pathway**

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Table 1 Supplementary Material.

A. The Specifications of the Pomegranate Extract (POX); B. Validation of the Analytical Method.

A.

Analytical data	Specifications	Methods
Punicalagins α and β	NLT 30% (w/w as db)	Internal method (HPLC)
Loss on Drying	NMT 8 %	Thermogravimetry
Total polyphenols	NLT 50 % (w/w as gallic acid equiv.)	Spectrophotometry (Folin-Ciocalteu)
Microbiology		
Total plate count (cfu/g)	NMT 1000	According to European Pharmacopoeia
Yeast and Mould (cfu/g)	NMT 100	
Enterobacterium (cfu/g)	NMT 100	
Salmonella (25 g)	Absence	
S. aureus (1 g)	Absence	
Escherichia coli (1 g)	Absence	

B.

Validation tests	Result	
Specificity	Noninterference	
Linearity	Correlation coefficient r^2	0.9999
	Slope (b)	10.995
	Intercept (a)	57.103
Repeatability	Average value (% w/w)	24.62
	Standard deviation (SD)	0.411
	Relative standard deviation (RSD %)	1.6685
Reproducibility	Average value (% w/w)	24.72
	Standard deviation (SD)	0.37
	Relative standard deviation (RSD %)	1.5110
Recovery	Average value (%)	101.27
	Standard deviation (SD)	0.92
	Relative standard deviation (RSD %)	0.9124

Table 2 Supplementary Material

Follow-up of Hematological Parameters

		RBC ($\times 10^6 / \text{mm}^3$)	HCT (%)	HGB (g/dL)	PLT ($\times 10^3 / \text{mm}^3$)	WBC ($\times 10^3 / \text{mm}^3$)
HC	Control					
	Baseline	5.1 \pm 0.4	24.1 \pm 2.7	8.3 \pm 0.7	313 \pm 20	9.9 \pm 1
	Experimental day	5.1 \pm 0.1	23.8 \pm 1.2	8.3 \pm 0.4	280 \pm 26	11.9 \pm 1
	POX					
	Baseline	4.9 \pm 0.1	22.3 \pm 1.3	7.8 \pm 0.6	324 \pm 29	12.9 \pm 2
	Experimental day	5.2 \pm 0.2	22.6 \pm 2.0	8.2 \pm 0.3	385 \pm 19	15 \pm 0.8
NC	Control					
	Baseline	4.8 \pm 0.5	24.3 \pm 2.0	7.7 \pm 0.6	340 \pm 42	13.9 \pm 3.5
	Experimental day	5.3 \pm 0.3	22.1 \pm 1.6	8.1 \pm 0.3	346 \pm 47	11.5 \pm 1.8
	POX					
	Baseline	4.7 \pm 0.4	24.7 \pm 1.5	8.4 \pm 0.5	304 \pm 25	14.1 \pm 1.5
	Experimental day	4.9 \pm 0.2	21.8 \pm 1.4	8.1 \pm 0.6	294 \pm 32	13.9 \pm 2.6

HC, hypercholesterolemic; NC, normocholesterolemic; POX, animals supplemented with pomegranate-fruit extract rich in punicalagins;

N = 6 animals/group.

Table 3 Supplementary Material

Follow-up of Heart Rate and Mean Arterial Pressure

			Heart rate (bpm)	Mean arterial pressure (mmHg)
HC	Control	Baseline	90±4	65±9
		Experimental day	87±3	75±7
	POX	Baseline	92±3	72±8
		Experimental day	88±4	75±8
NC	Control	Baseline	95±5	67±6
		Experimental day	91±4	71±5
	POX	Baseline	95±4	71±7
		Experimental day	89±4	70±5

bpm, beats per minute; HC, hypercholesterolemic; NC, normocholesterolemic; POX, animals supplemented with pomegranate-fruit extract rich in punicalagins.

N = 6 animals/group.