Unconjugated Bilirubin exerts Pro-Apoptotic Effect on Platelets *via* p38-MAPK activation

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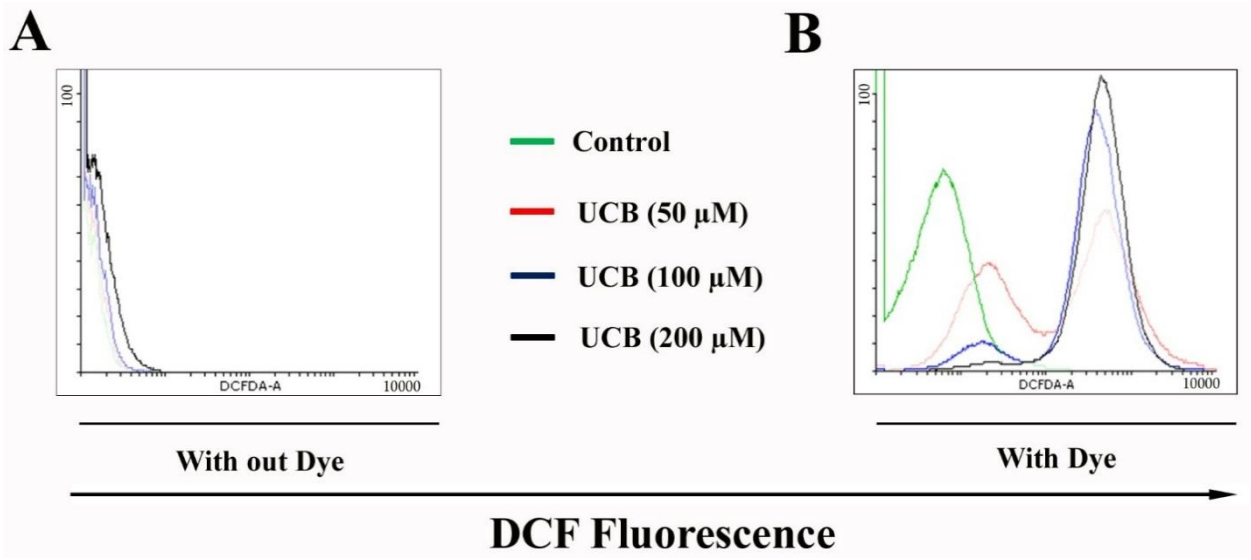
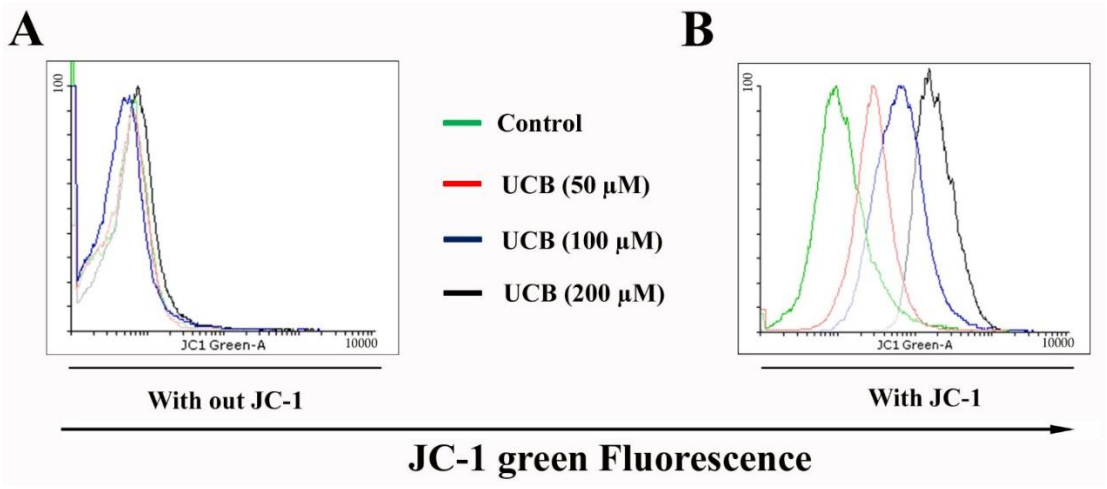
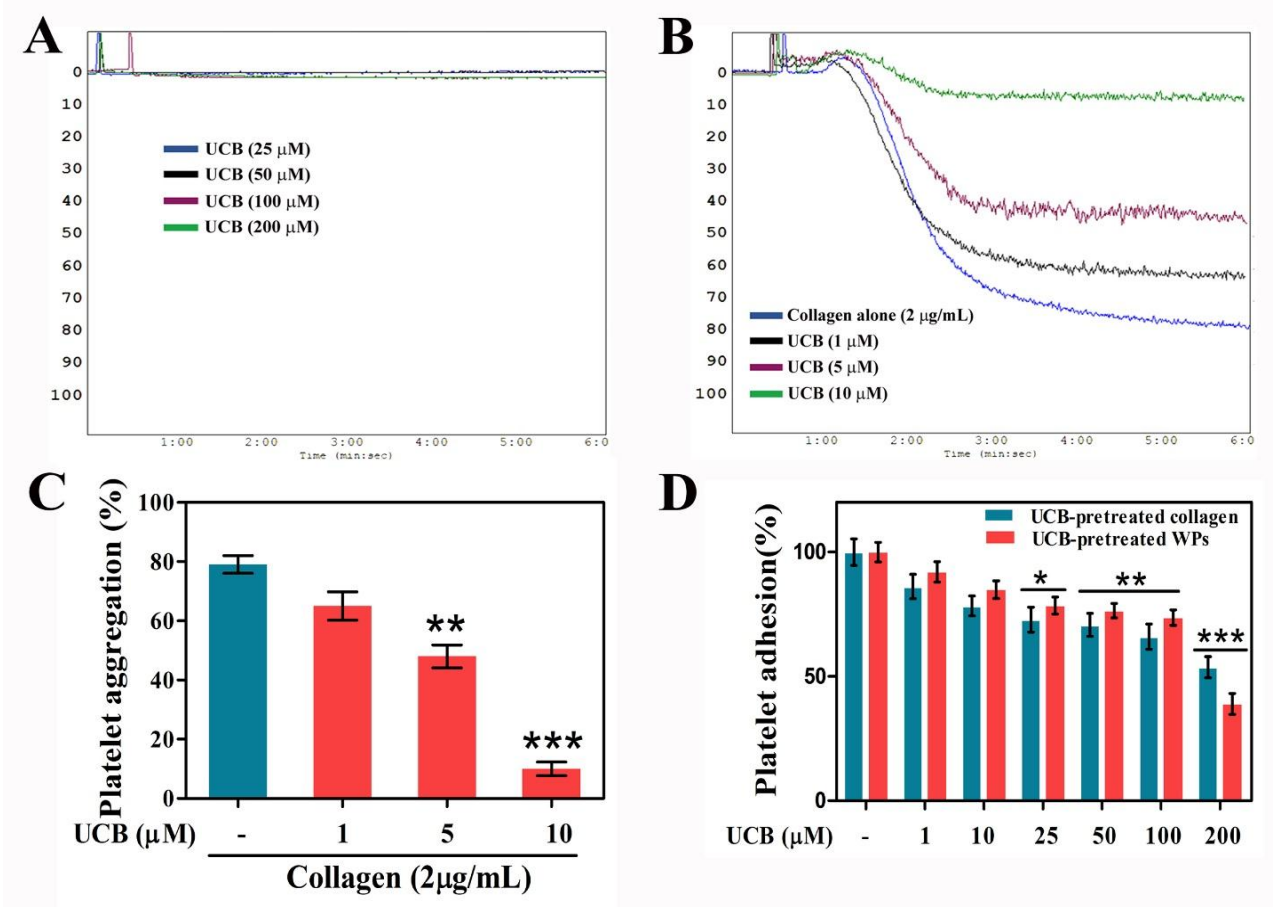
Supplementary information



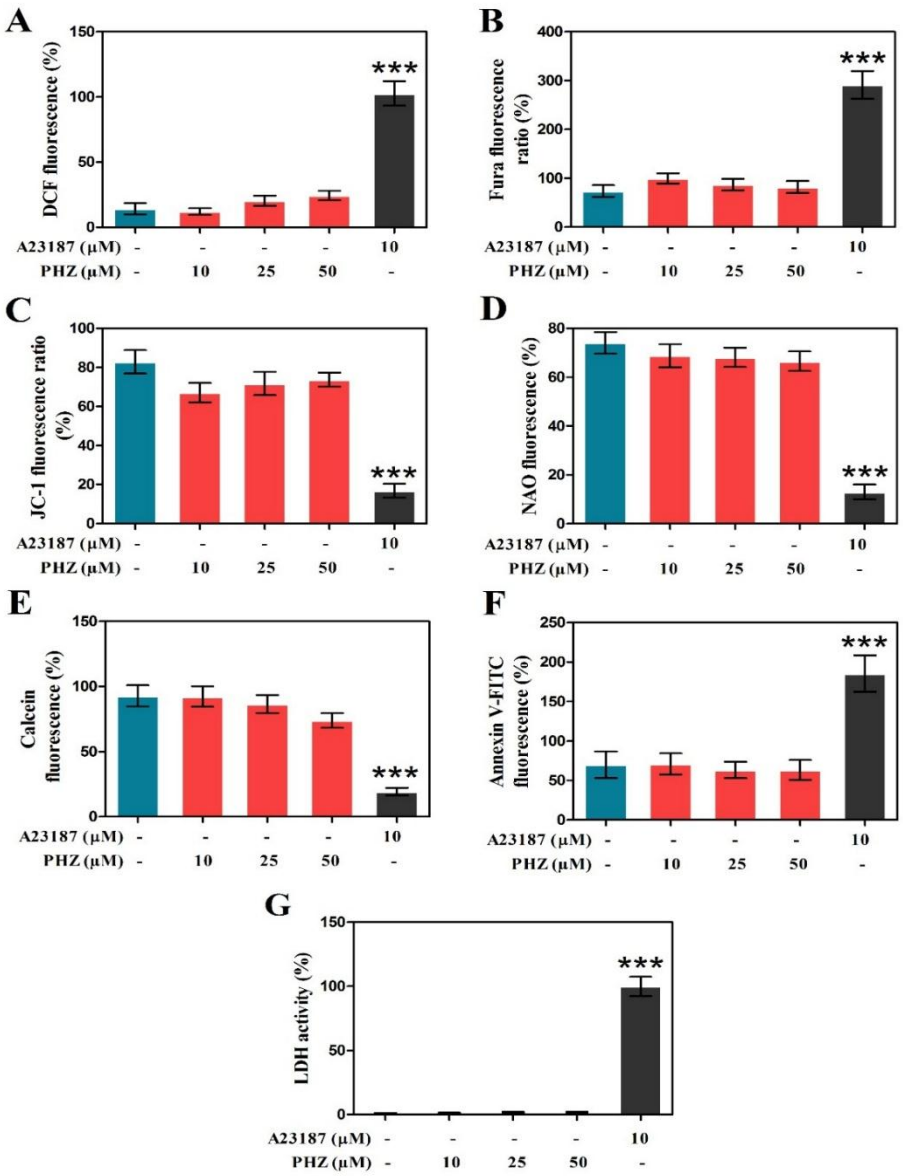
Supplementary Figure 1: FACS analysis of UCB treated platelets in presence/absence of DCFDA. Flow cytometric analysis of UCB treated platelets (**A**) in absence of DCFDA and (**B**) in presence of DCFDA.



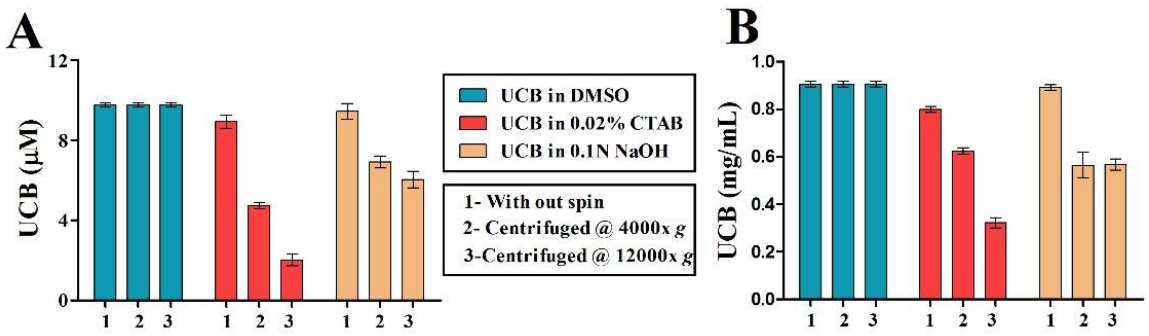
Supplementary Figure 2: FACS analysis of UCB treated platelets in presence/absence of JC-1. Flow cytometric analysis of UCB treated platelets (**A**) in absence of JC-1 and (**B**) in presence of JC-1.



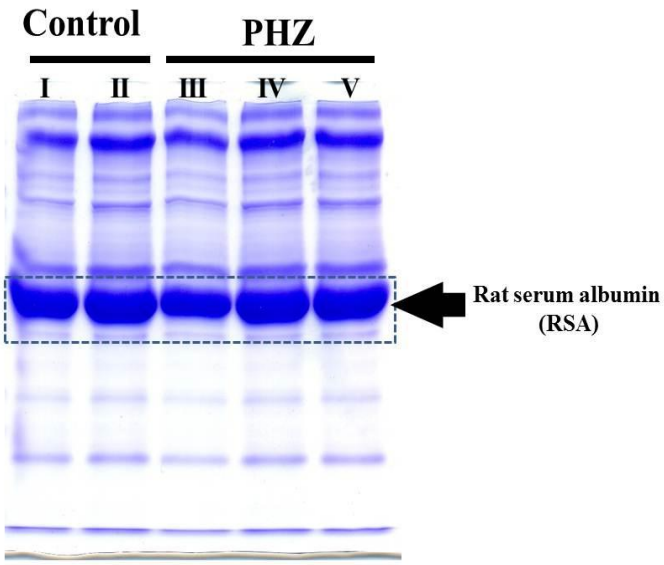
Supplementary Figure 3: Effect of UCB on platelet aggregation and adhesion. Effect of UCB on platelet aggregation induced by **(A)** UCB alone and **(B)** Collagen. **(C)** Graphical representation of percentage platelet aggregation with collagen (2µg/mL) in presence or absence of UCB. **(D)** Effect of UCB on platelet adhesion on immobilized collagen type I. $p^{*} < 0.05$, $p^{**} < 0.01$, $p^{***} < 0.001$; significant compared to control platelets.



Supplementary Figure 4: Effect of PHZ on platelet apoptotic markers *in vitro*. Platelets were treated with the dose that corresponds to the concentration of PHZ in circulation during *in vivo* administration of PHZ and the following markers were determined. **(A)** ROS, **(B)** intracellular calcium levels, **(C)** mitochondrial membrane potential, **(D)** cardiolipin peroxidation, **(E)** mPTP formation and **(F)** PS externalization. Values are presented as mean ± SEM (n=5), and expressed as percentage increase/decrease in fluorescence. **(G)** LDH activity. $p^{***} < 0.001$; significant compared to A23187 treated platelets.



Supplementary Figure 5: Determination of the concentration of bilirubin. The concentration of bilirubin dissolved in different solvents was estimated by **(A)** spectrophotometry and **(B)** bilirubin estimation kit.



Supplementary Figure 6: Full length image of SDS-PAGE from figure 7. Regions of interest are highlighted and are presented as cropped image in figure 7. Samples are as follows; Lane I & II- control group serum, Lane III, IV & V- PHZ treated group serum.

Supplementary Table 1. Clinical characteristics of HS and HB patients.

Characteristics		HS	HB
	Total subjects	n = 21	n = 35
	Gender (Males/Females)	16/05	29/06
PLT count ($x10^3$/µL)	Total subjects	327.61 ± 39.37	148.14 ± 52.65
	Male	330.56 ± 42.30	142.62 ± 55.15
	Female	318.20 ± 25.84	172.50 ± 24.85
UCB concentration (mg/dL)	Total subjects	0.40 ± 0.21	1.57 ± 1.41
	Male	0.42 ± 0.22	1.62 ± 1.19
	Female	0.33 ± 0.13	2.17 ± 1.91

Information in the above table were represented as follows: Data presented for Platelets as mean count ± SD; UCB as mean concentration in mg/dL ± SD