The modulatory effect of pomegranate mesocarp on ribose-glycated protein

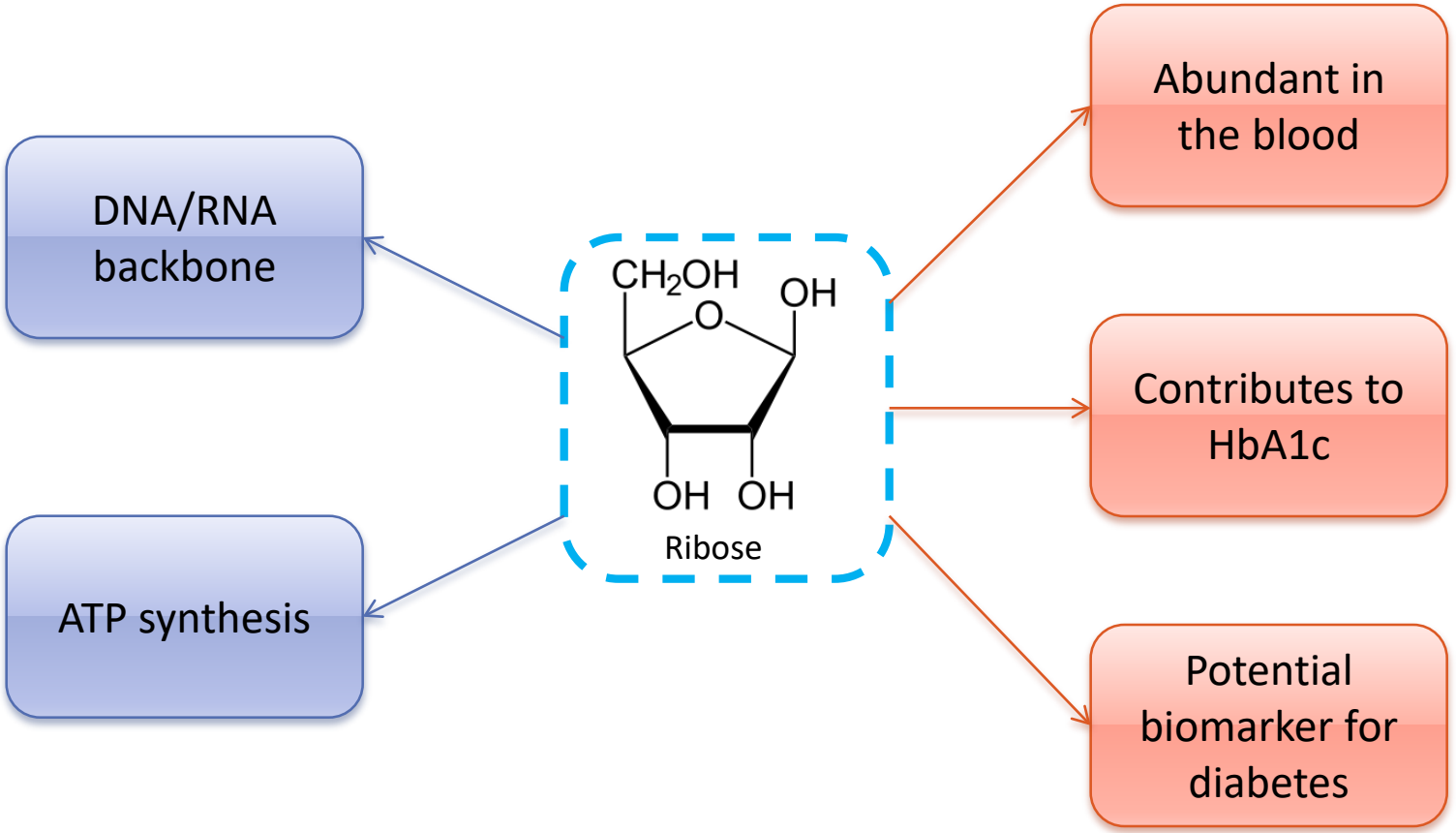
Piteesha Ramlagan[1], Vidushi Neergheen-Bhujun[1], Emmanuel Bourdon[2], Theeshan Bahorun[3]

[1] Department of Health Sciences, Faculty of Science and ANDI Center for Biomedical and Biomaterials Research University of Mauritius

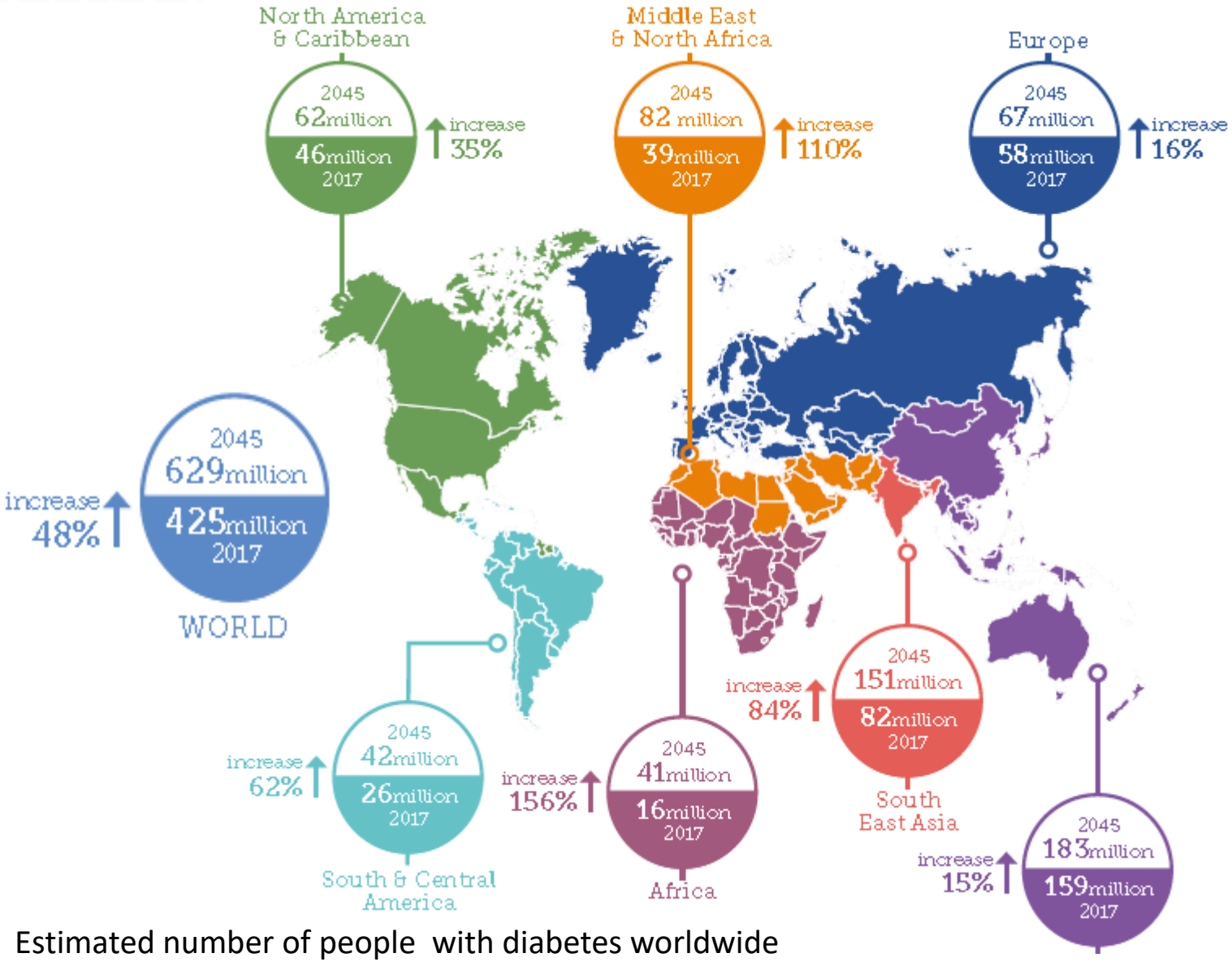
[2] Université de La Réunion, INSERM, UMR 1188 Diabète athérothombose Thérapies Réunion Océan Indien (DéTROI), plateforme CYROI, Saint-Denis de La Réunion,France

[3] ANDI Center for Biomedical and Biomaterials Research, University of Mauritius

Introduction: Ribose



Introduction: Diabetes Statistics



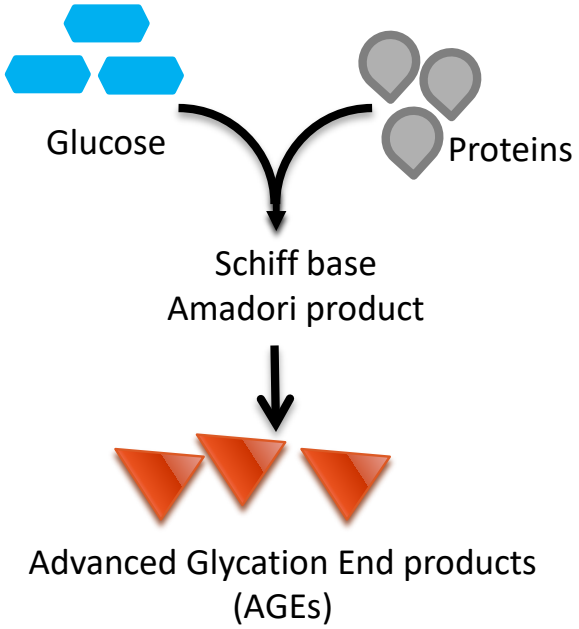
Estimated number of people with diabetes worldwide

(IDF Diabetes Atlas, 2017)

Introduction: Diabetes Statistics

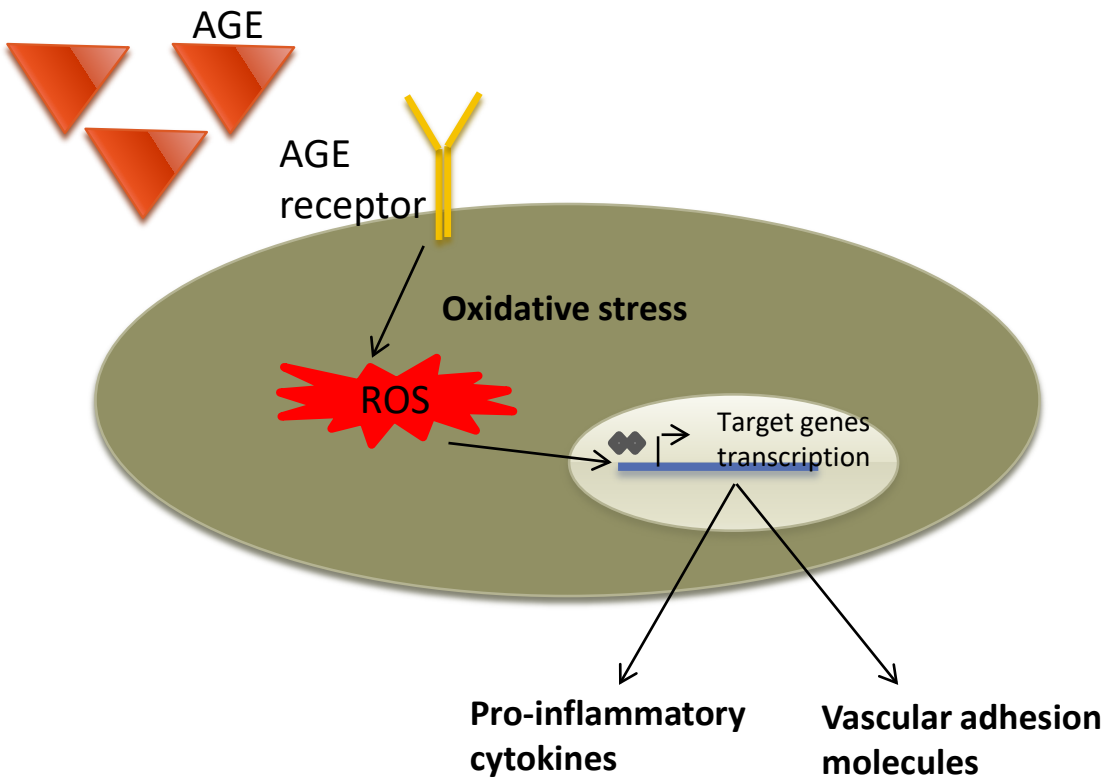
Diabetes estimates (20-79 years)	Mauritius	Reunion	Madagascar	Comoros
Prevalence, %	22	13.8	3.9	11.9
% of diabetic population	17.9	12.9	1.45	3.83
Diabetes related deaths	2609.3	NA	4685.6	339.7
Mean diabetes related expenditure (USD), per person	535	NA	27	100

Introduction: glycation



Introduction: AGEs & diabetic complications

AGE receptor: CD36

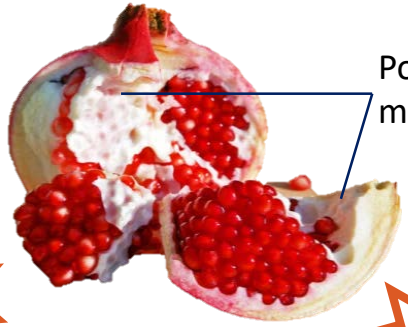


- Over-expressed by AGEs
- Insulin resistance
- Pro-atherogenic

Diabetic complications

Introduction: pomegranate

- Functional food with anti-diabetic potential
- Non-edible parts bioactive



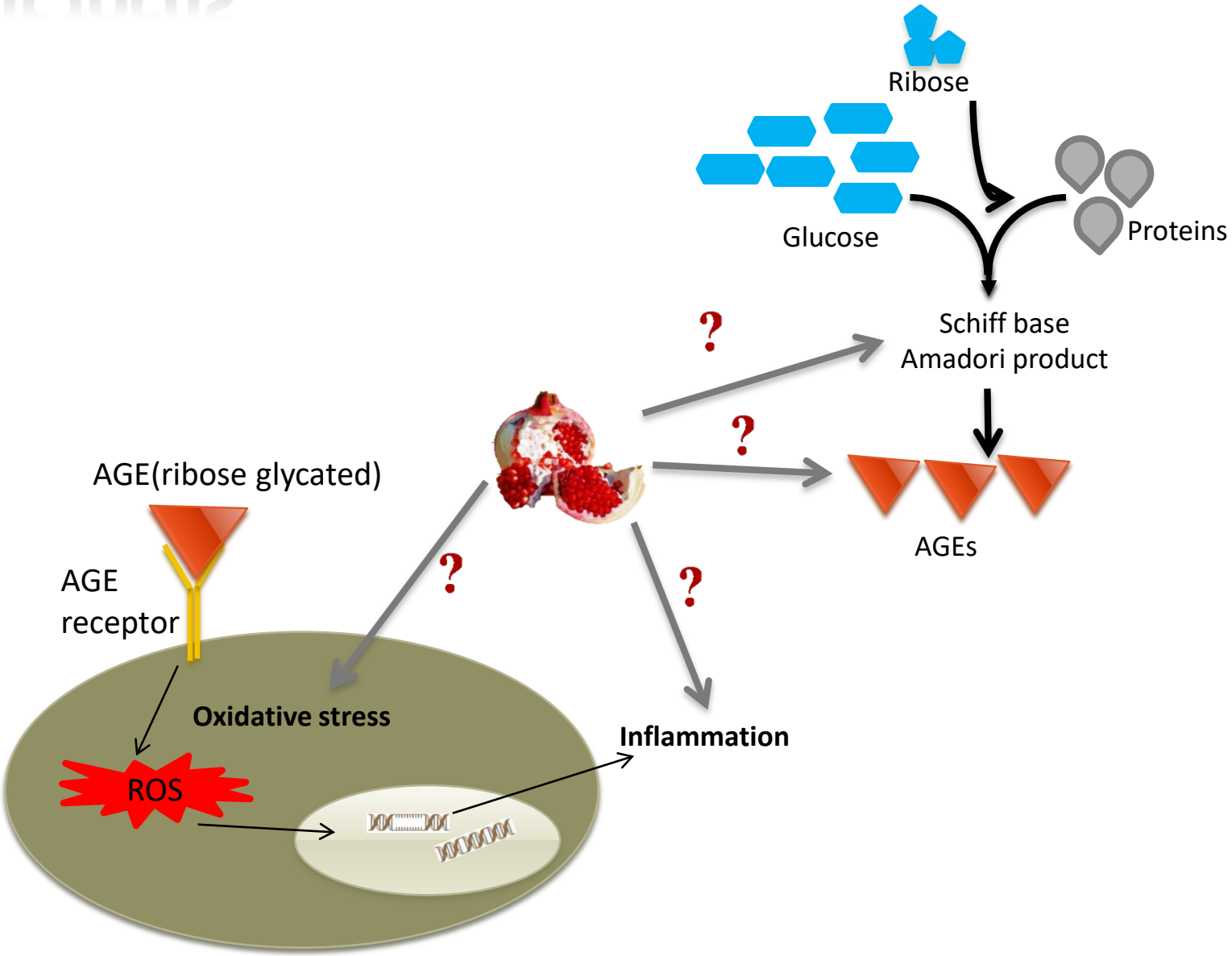
Polyphenolic richness

Antioxidant potential

Anti-inflammatory capacities

Suppresses CD36 expression

Our focus


Ribose
Glucose
Proteins
Schiff base
Amadori product
AGEs
AGE(ribose glycated)
AGE
receptor
Oxidative stress
ROS
Inflammation
?
?
?
?

Anti-diabetic potencies of PME

Freeze dried and ground. Exhaustively extracted by 70% methanol for 3 days. Lyophilised.

Pomegranate mesocarp extract (PME)

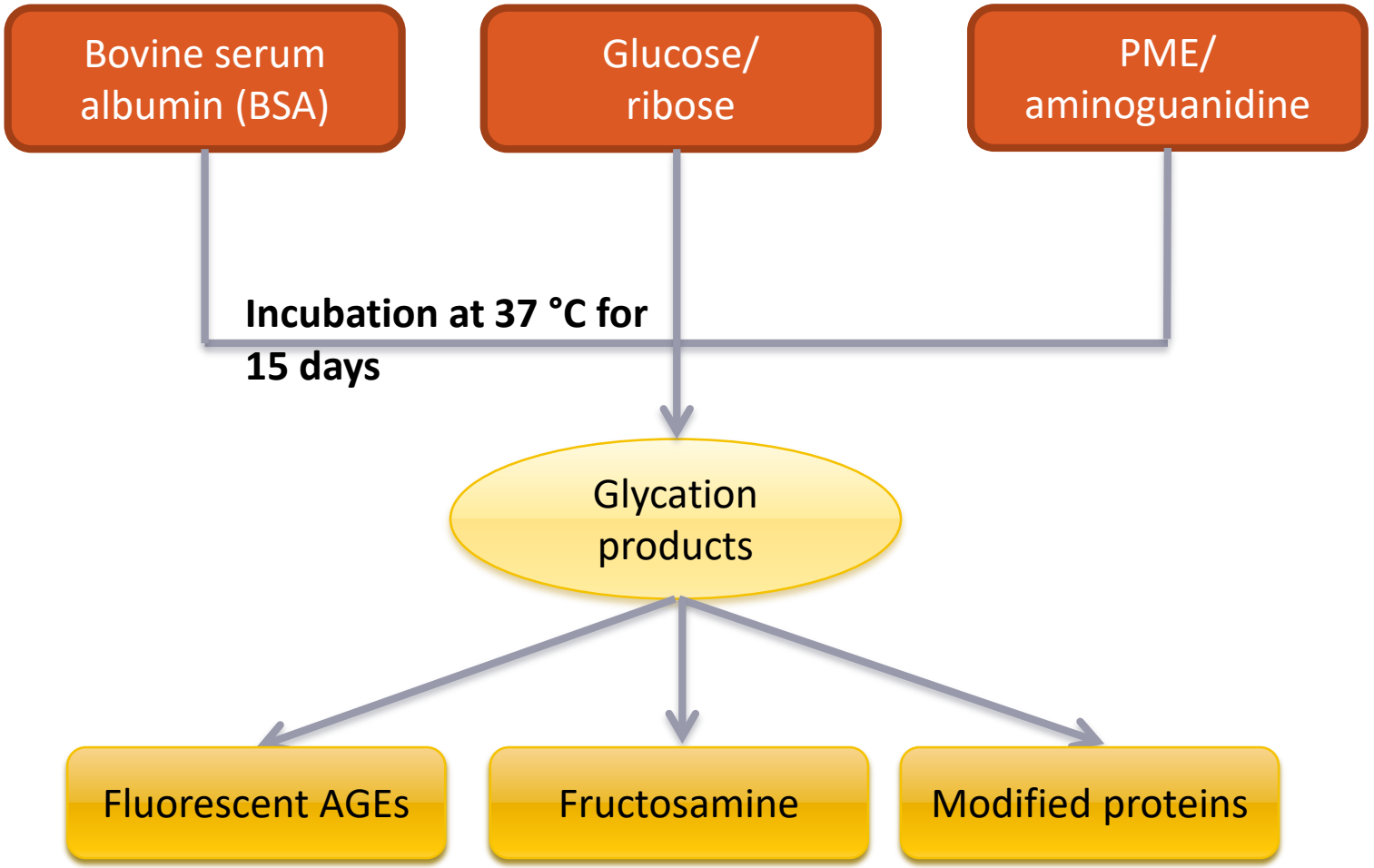
Anti-glycation assays

Fluorescent AGEs
Fructosamine
Protein carbonyl
AOPP

In vitro diabetic model

Cell viability
ROS
Protein carbonyl
IL-6

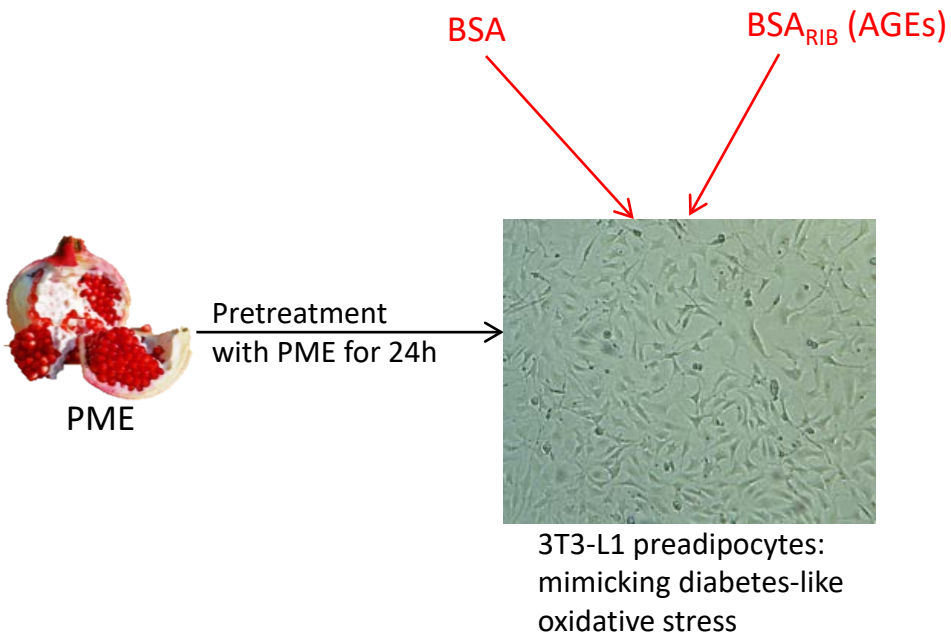
Anti-glycative activity of PME



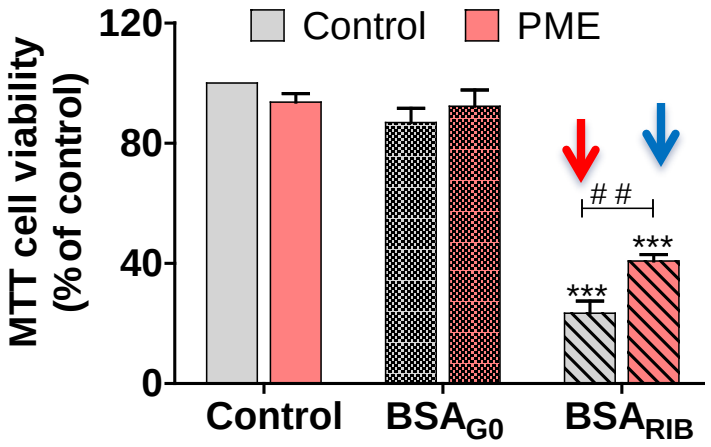
Anti-glycative activity of PME

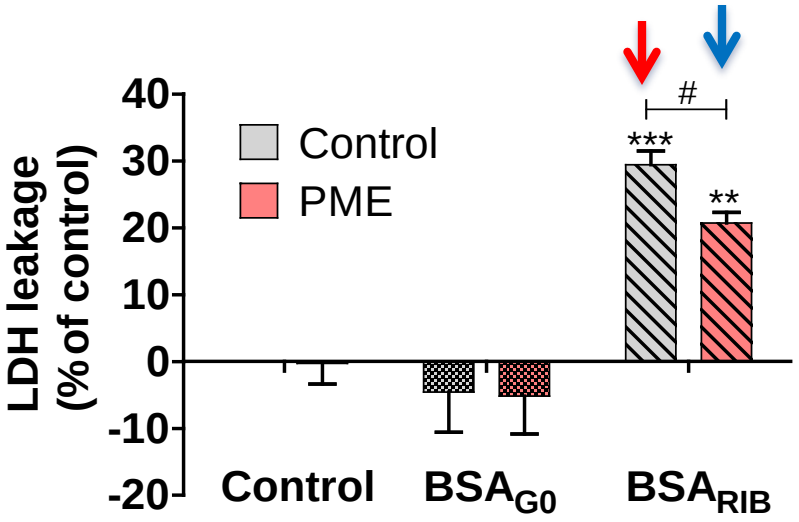
Experimental group	Level of			
	Fluorescent AGEs (% BSA)	*Fructosamine (mM DMF)*	*Protein carbonyl (% BSA)*	*AOPP (nmol chloramine-T/mg protein*
BSA	100	0.711 ± 0.10	100	0.07 ± 0.01
BSA + GLU+				
DMSO	176.21 ± 2.32***	4.97 ± 0.28***	107.29 ± 5.13	0.33 ± 0.05
PME	102.15 ± 0.49 ###	2.99 ± 0.29$^{***}_{\#\#}$	100.26 ± 11.62	0.35 ± 0.08
Aminoguanidine	103.46 ± 0.60 ###	3.49 ± 0.26$^{***}_{\#}$	94.61 ± 5.21	0.36 ± 0.03
BSA + RIB +				
DMSO	1346.69 ± 20.82***	4.86 ± 0.15***	175.01 ± 6.14***	3.91 ± 0.18***
PME	103.36 ± 12.36 ###	3.14 ± 0.35$^{***}_{\#\#\#}$	99.82 ± 9.23 ###	1.48 ± 0.27$^{***}_{\#\#\#}$
Aminoguanidine	114.85 ± 18.10 ###	3.12 ± 0.25$^{***}_{\#\#\#}$	131.85 ± 5.94$^{*}_{\#\#}$	2.55 ± 0.14$^{***}_{\#\#\#}$

Effect of PME in *in vitro* diabetic model

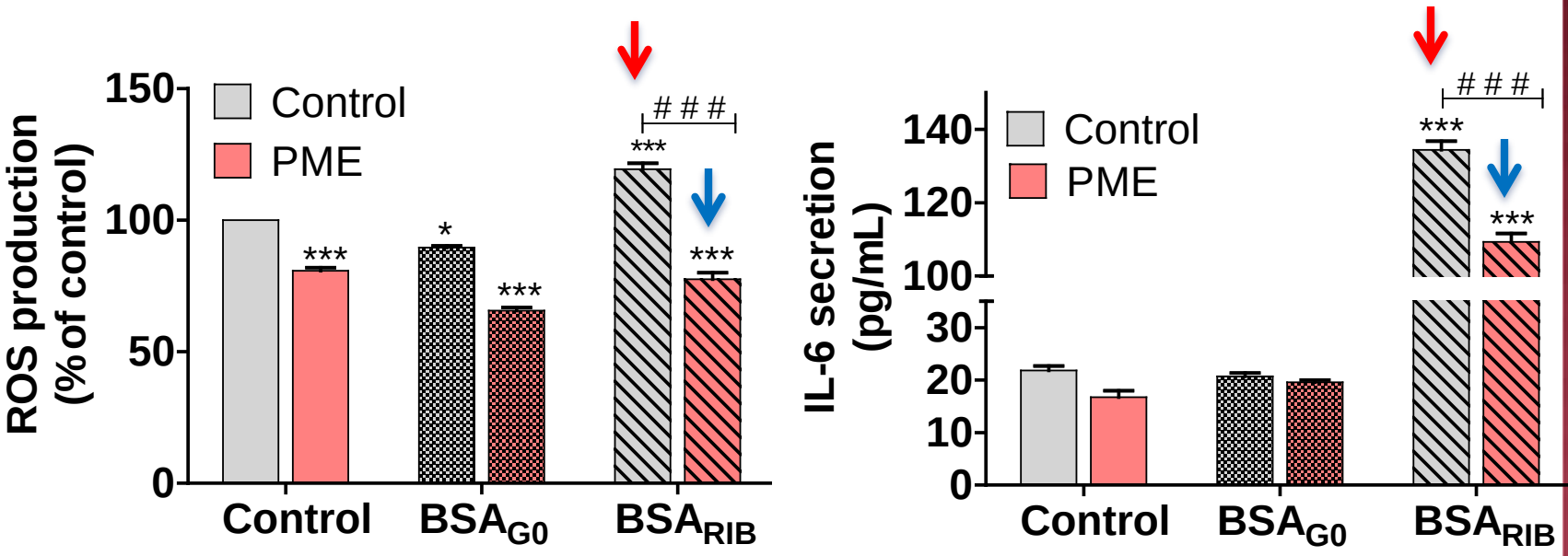


PME protects against AGEs-induced cell death

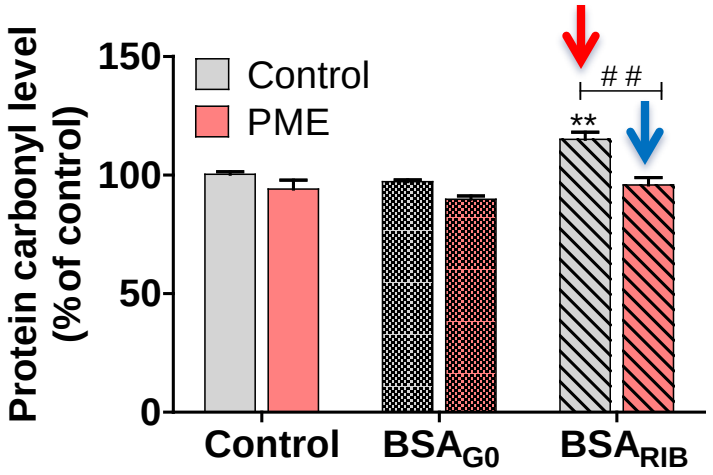


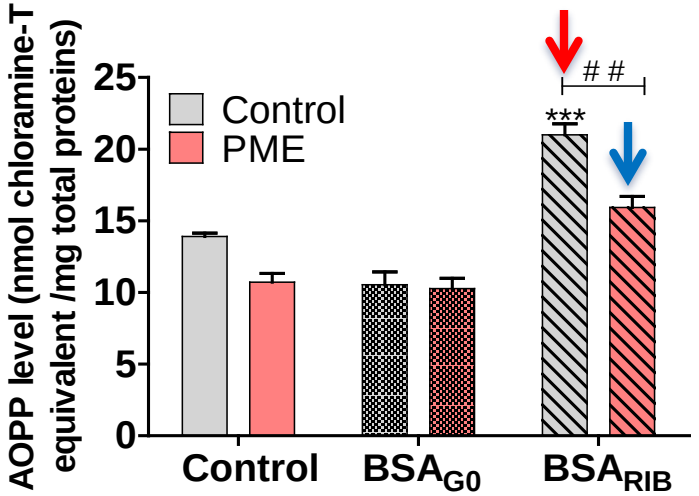


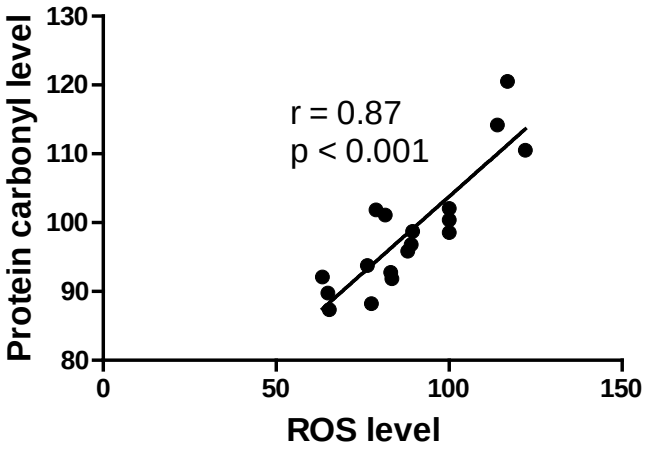
PME counteracts AGEs-induced oxidative stress and inflammation

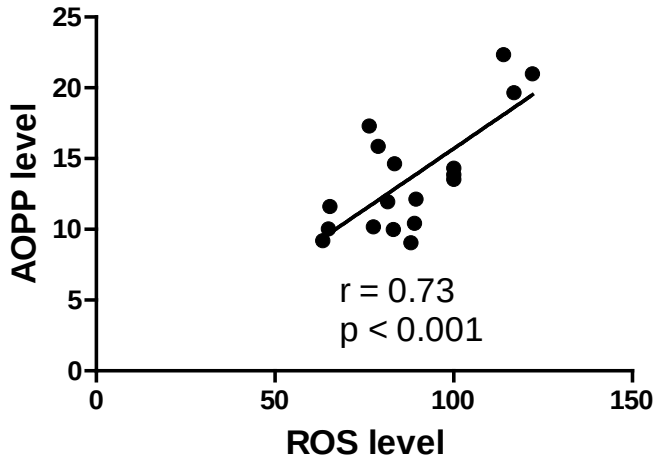


PME lowers accumulation of oxidatively modified proteins









Fractionation of PME

Pomegranate extract (PME)

Dissolved in water and partitioned in dichloromethane

- DCM fraction
- Aqueous phase

Partitioned in ethyl acetate

- EtAc fraction
- Aqueous phase

Partitioned in *n*-butanol

- BUT fraction
- Water residue

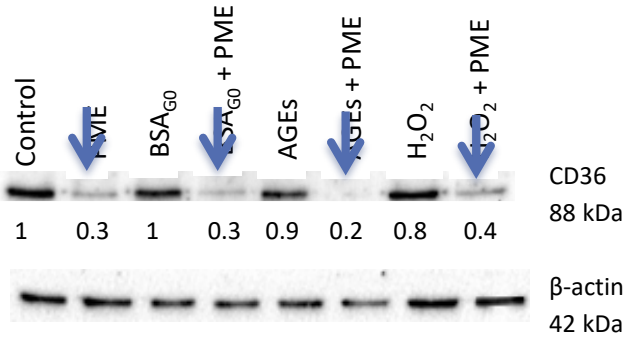
Polyphenolic content of pomegranate mesocarp fractions

Fraction	Total phenolic content	Total flavonoid content	Hydrolysable tannin content
Crude	483.97 ± 18.32 [b]	383.78 ± 9.20 [c]	704.52 ± 7.74 [d]
Dichloro-methane	80.48 ± 2.85 [e]	18.04 ± 0.34 [e]	127.61 ± 7.09 [e]
Ethyl acetate	570.14 ± 14.13 [a]	533.85 ± 12.02 [b]	901.46 ± 11.64 [b]
n-butanol	323.27 ± 6.40 [d]	681.75 ± 5.97 [a]	945.69 ± 12.86 [a]
Water residue	431.78 ± 8.01 [c]	350.25 ± 14.71 [d]	739.08 ± 3.89 [c]

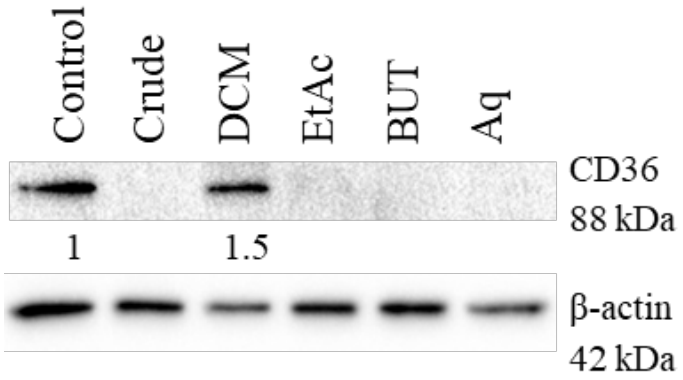
Antioxidant activity of pomegranate mesocarp fractions

Fraction	IC_{50} value (µg/mL) for	
	ABTS radical scavenging	DPPH radical scavenging
Crude	2.340 ± 0.017 [a]	3.396 ± 0.341 [a]
Dichloro-methane	39.732 ± 1.752 [b]	88.353 ± 8.904 [b]
Ethyl acetate	1.638 ± 0.081 [a]	2.962 ± 0.190 [a]
n-butanol	1.181 ± 0.042 [a]	2.035 ± 0.335 [a]
Water residue	2.574 ± 0.177 [a]	3.575 ± 0.462 [a]

Pomegranate mesocarp fractions modulate CD36 expression

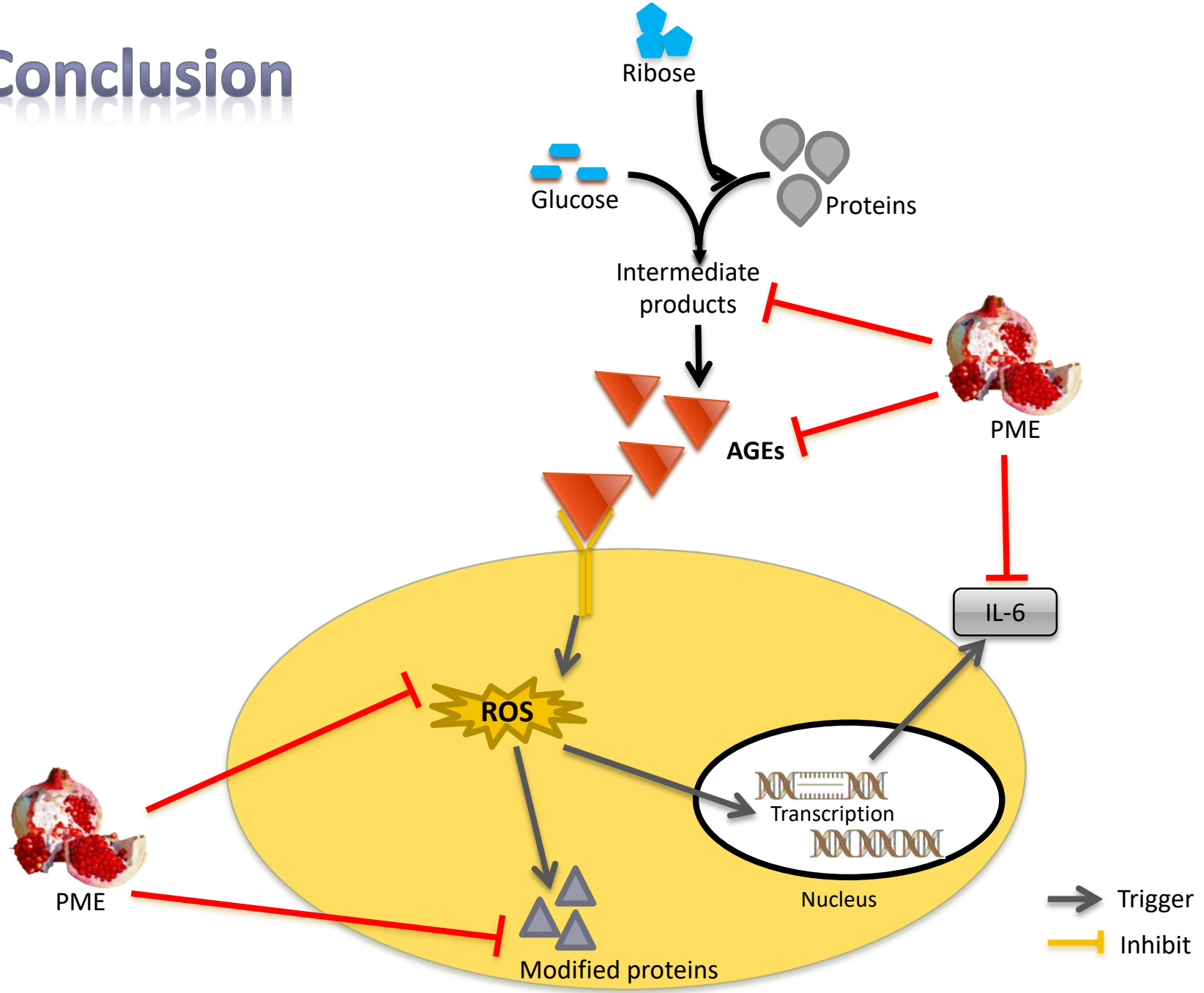


Effect of PME on CD36 protein expression
(Densitometry values are expressed relative to control and normalized against β-actin)

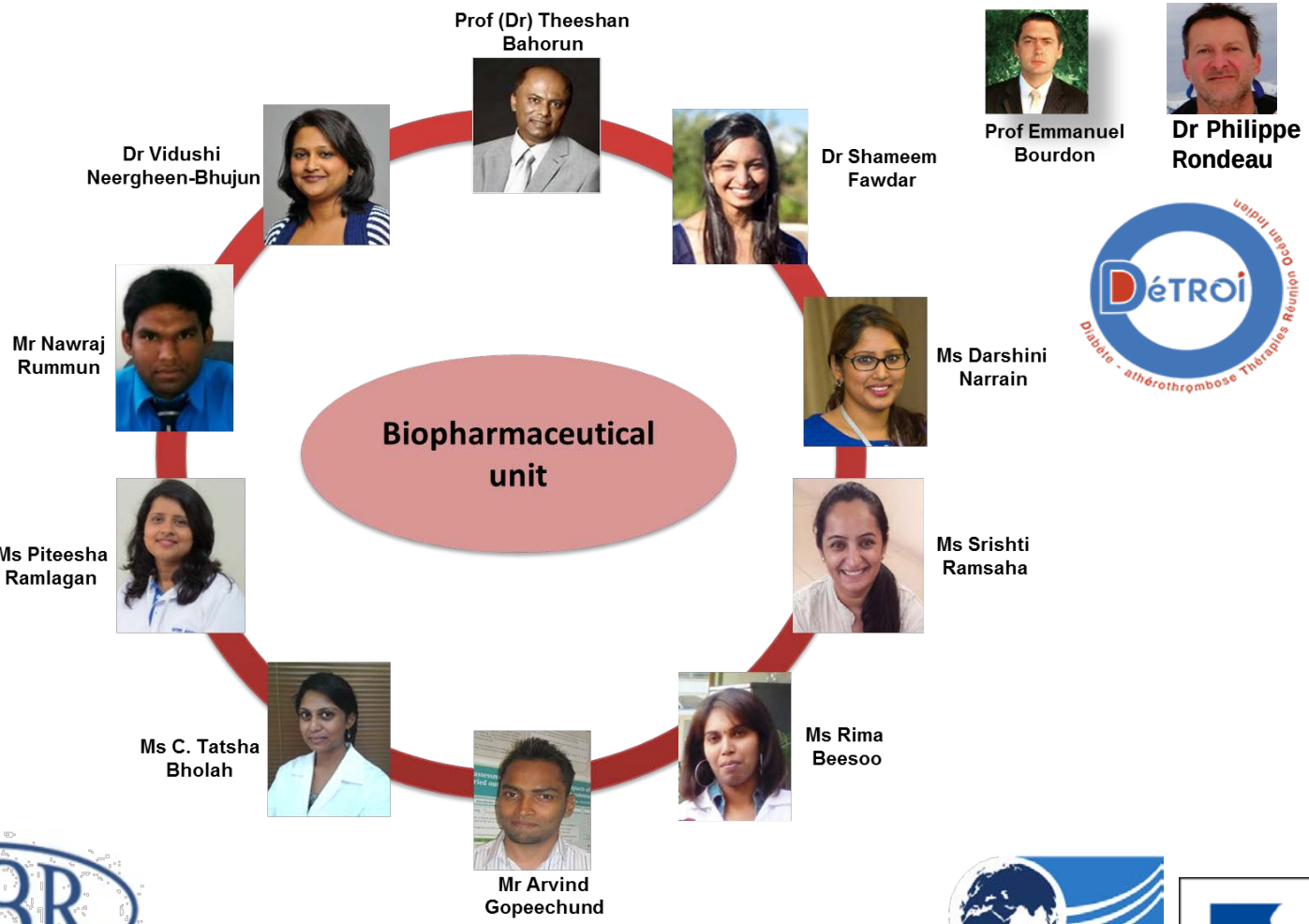


Effect of crude and fractions of mesocarp on CD36 protein expression from preadipocytes
Densitometry values are expressed relative to control and normalized against β-actin.

Conclusion


Ribose
Glucose
Proteins
Intermediate products
PME
AGEs
IL-6
ROS
Transcription
Nucleus
PME
Modified proteins
Trigger
Inhibit

Acknowledgement



CBBR

MRC

UNIVERSITY OF MAURITIUS

QualiREG
La qualité pour le développement en océan Indien

UNIVERSITÉ DE LA RÉUNION
S'ouvrir aux mondes

CYROI
Recherche Santé Bio-innovation

Liberté • Égalité • Fraternité
RÉPUBLIQUE FRANÇAISE

MINISTÈRE DE L'ENSEIGNEMENT SUPÉRIEUR ET DE LA RECHERCHE

Thank you

Merci

Misaotra anao

Marahaba