# Pomegranate mesocarp: a novel protective role against diabetes


Presented by

**RAMLAGAN Piteesha**

*MPhil/PhD Student*

*Centre for Biomedical and Biomaterials Research*

*University of Mauritius*

*Mauritius*



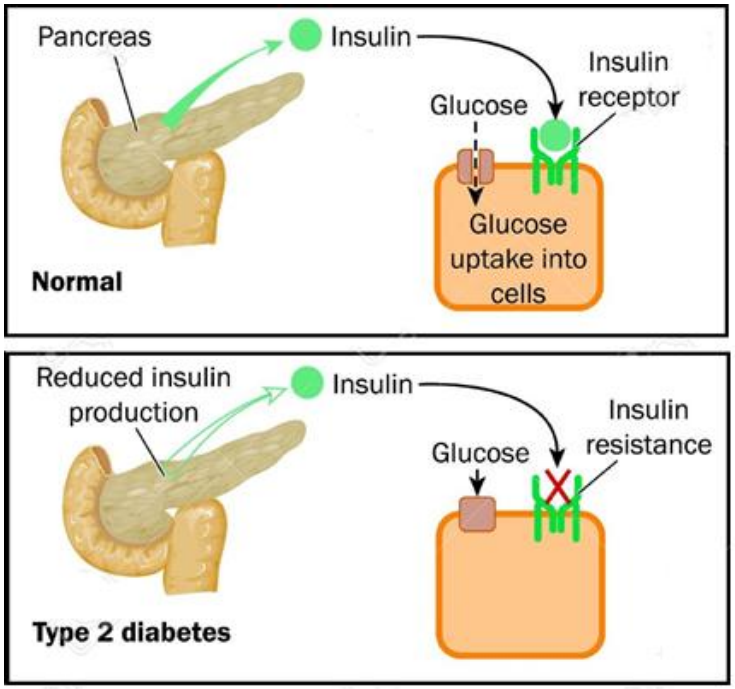
Rencontres de l'Agro-alimentaire en Océan Indien

28th Nov -2nd Dec 2016

Saint Pierre, La Réunion

# Type II diabetes

- Worldwide clinical disorder




- Increasing deaths due to diabetic complications

# Diabetic complications

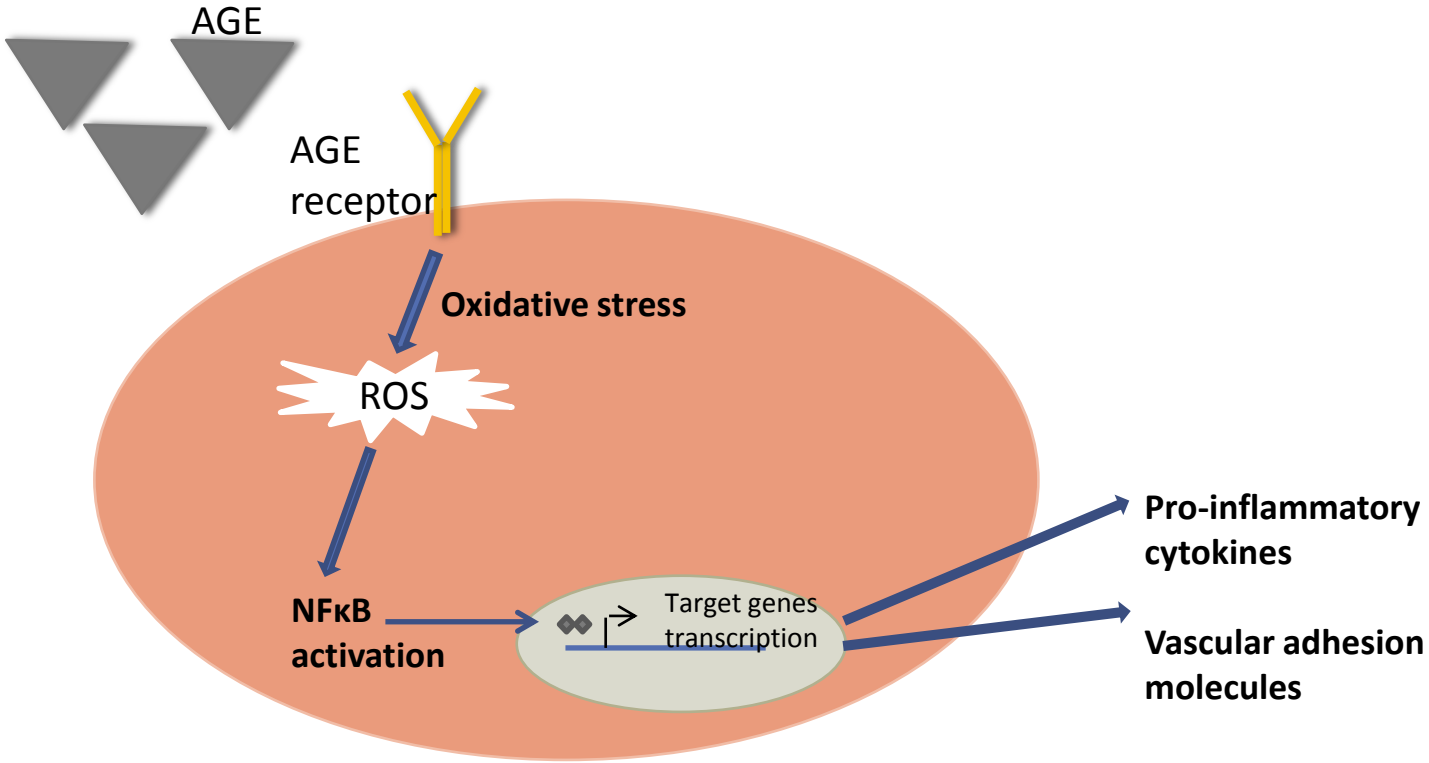
Hyperglycemia induces Glycation

Sugar ($C_6H_{12}O_6$) + $H_2N$-Protein → Advanced Glycation End Products (AGEs)

# Diabetic complications

## Hyperglycemia induces Glycation

Sugar ($C_6H_{12}O_6$) + $H_2N$-Protein → Advanced Glycation End Products (AGEs)



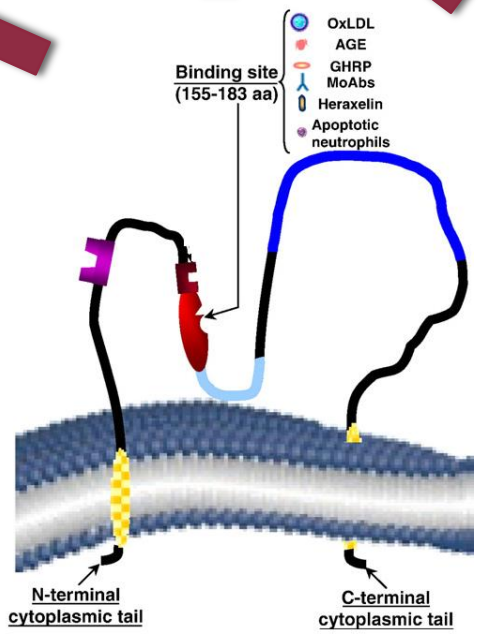

# CD36: an AGE receptor

Implicated in insulin resistance, dyslipidemia

Down-regulates leptin expression

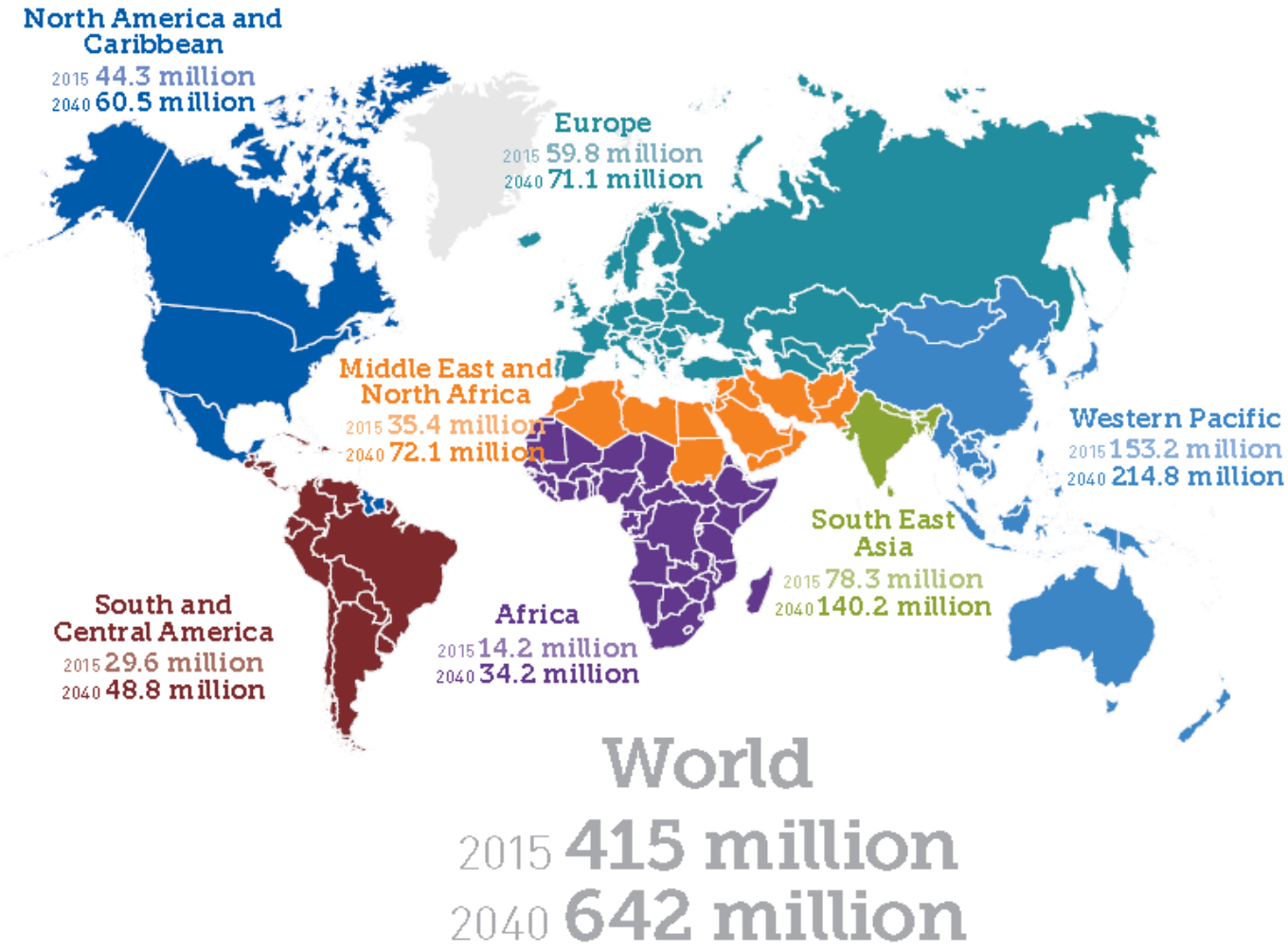
Atherosclerotic

Overexpressed in presence of AGEs




3D representation of CD36 receptor on cellular membrane (Collot-Teixeira *et al*., 2007)

# Diabetes Statistics




Estimated number of people with diabetes worldwide

(IDF Diabetes Atlas, 2015)

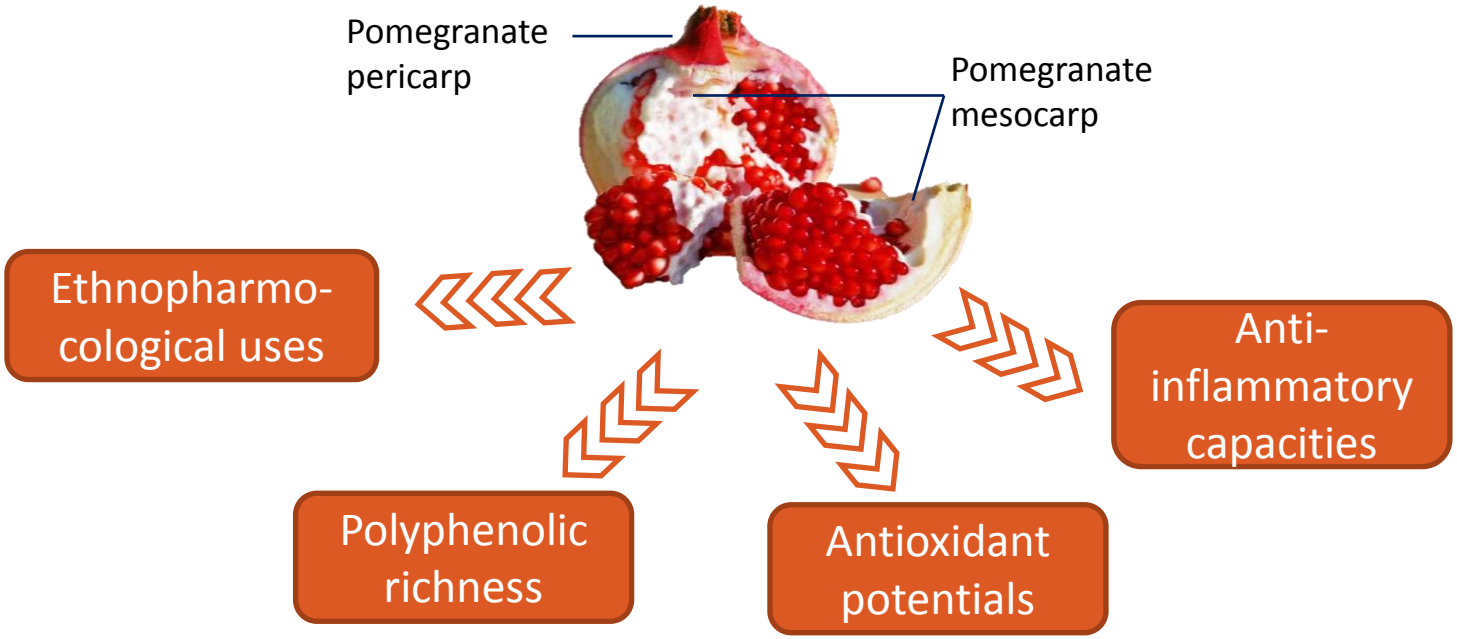
# Diabetes Statistics

| Diabetes estimates (20-79 years) | Mauritius | Reunion | Madagascar | Comoros | South Africa |
|---|---|---|---|---|---|
| Prevalence, % | 22.3 | 15.8 | 4.0 | 9.9 | 7.6 |
| % of diabetic population | 18.3 | 13.0 | 1.5 | 3.6 | 4.2 |
| Diabetes related deaths | 2931.2 | NA | 5580.2 | 318.7 | 57 318.6 |
| Mean diabetes related expenditure (USD), per person | 934.3 | NA | 111.4 | 152.2 | 1736.1 |

# Pomegranate

- Antioxidant functional foods with anti-diabetic and anti-atherogenic potentials

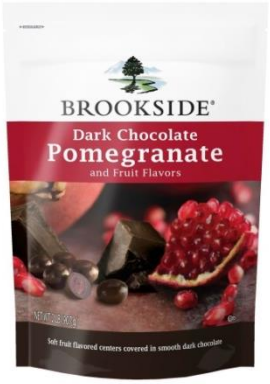
- Non-edible parts bioactive in multi-assay antioxidant systems



# Uses of pomegranate fruit

**Food**

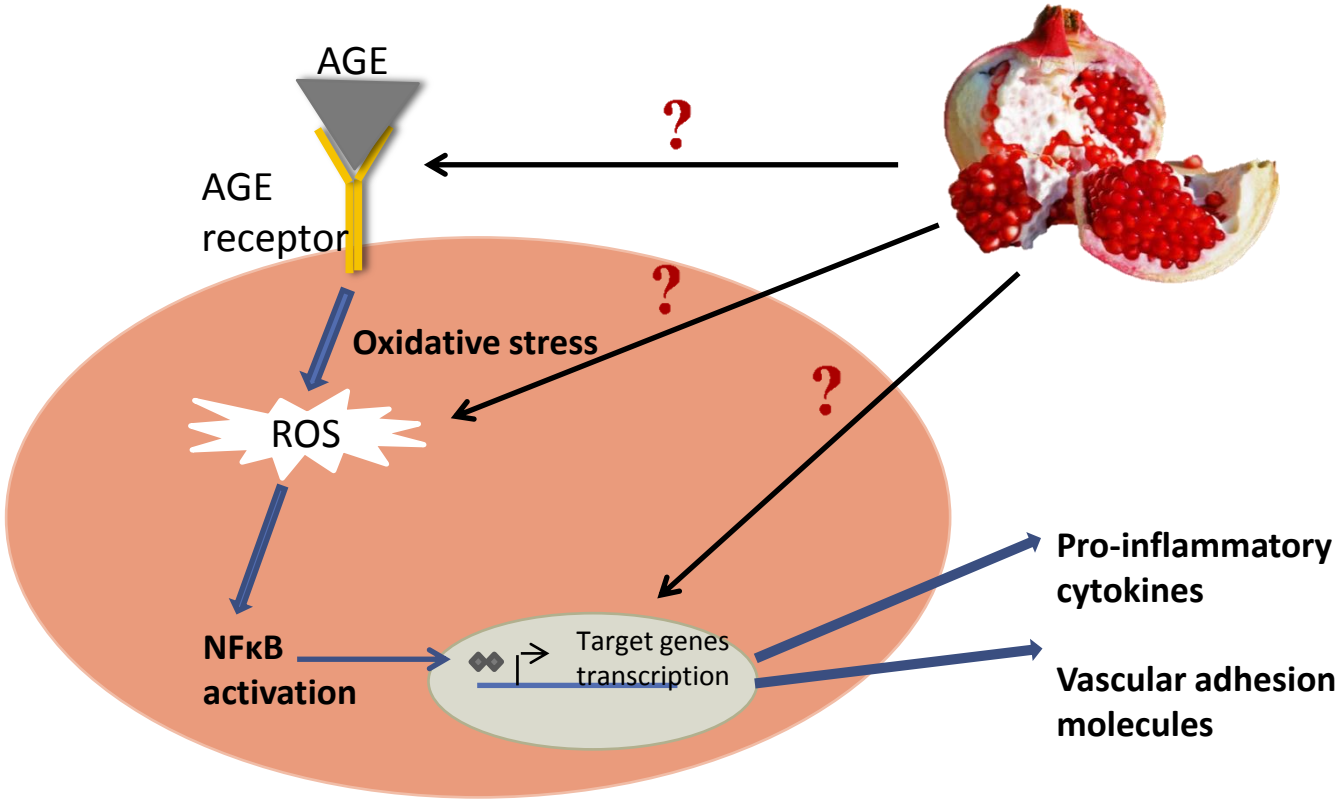
**Cosmetics**





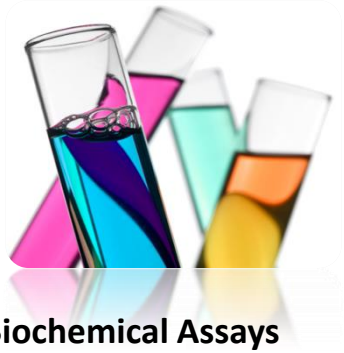

# Our focus


AGE
AGE receptor
?
?
?
Oxidative stress
ROS
NFκB activation
Target genes transcription
Pro-inflammatory cytokines
Vascular adhesion molecules

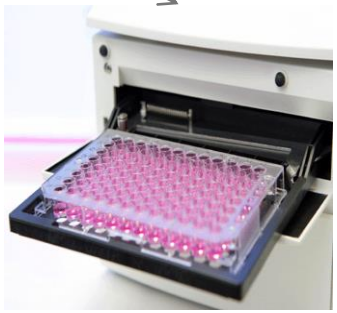
# Biochemical and antioxidant screening

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

**Pomegranate extract (PME)**



**Biochemical Assays**
(Phenolic, Flavonoid, Proanthocyanidin, hydrolyzable tannin)



**Antioxidant Assays**
(FRAP, ORAC, Iron (II) chelating, Superoxide, nitric oxide, hydroxyl, ABTS, DPPH scavenging)

# Rich in polyphenolics

- ✓ Phenolic content: **416.1 ± 11.4** mg GAE/g lyophilised powder (LP)
- ✓ Flavonoid content: **310.6 ± 9.1** mg QE/g LP
- ✓ Hydrolysable tannin content: **699.4 ± 16.5** mg TAE/g LP
- ✓ Proanthocyanidin content: **1.6 ± 0.1** mg CCE/g LP

# High antioxidant capacities

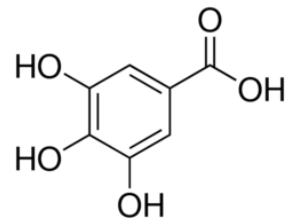
**Antioxidant capacities:**

| | PME | Green tea | Black Tea | Gallic acid |
|---|---|---|---|---|
| **FRAP value (mmol)** | $12.7 \pm 1.1^{b}$ | $6.0 \pm 0.5^{b}$ | $4.4 \pm 0.3^{b}$ | $45.1 \pm 4.5^{a}$ |
| **ORAC value (mmol)** | $1.1 \pm 0.1^{b}$ | $4.0 \pm 0.2^{b}$ | $2.2 \pm 0.1^{b}$ | $17.7 \pm 2.6^{a}$ |

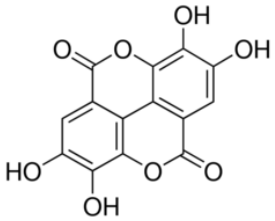
***In vitro*** **antioxidant activities of PME and standard antioxidant**

| In vitro antioxidant activity | $IC_{50}$ of PME (µg/mL) | $IC_{50}$ of gallic acid (µg/mL) |
|---|---|---|
| **Nitric oxide radical scavenging** | $1.16 \pm 0.03^{a}$ | $88.16 \pm 2.84^{b}$ |
| **ABTS radical scavenging** | $2.74 \pm 0.07^{b}$ | $0.53 \pm 0.02^{a}$ |
| **DPPH radical scavenging** | $7.30 \pm 0.26^{b}$ | $1.45 \pm 0.11^{a}$ |
| **Superoxide radical scavenging** | $18.77 \pm 0.69^{b}$ | $6.48 \pm 0.24^{a}$ |
| **Hydroxyl radical scavenging** | $28.29 \pm 1.11^{a}$ | $173.89 \pm 7.18^{b}$ |
| **Iron (II) chelating** | $34.12 \pm 1.66^{a}$ | $5453.53 \pm 191.54^{b}$ |

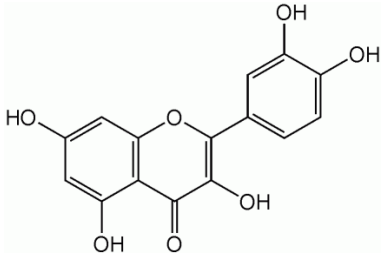
# High antioxidant capacities



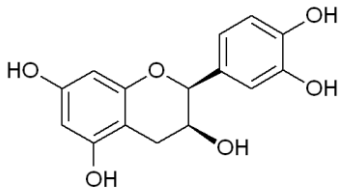
Gallic acid



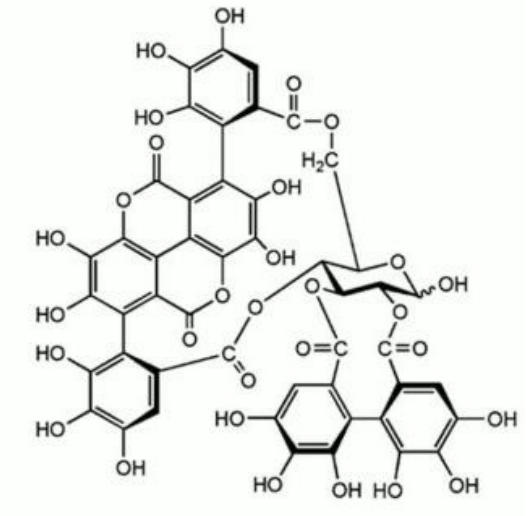
Ellagic acid



Quercetin



Catechin



Punicalagin

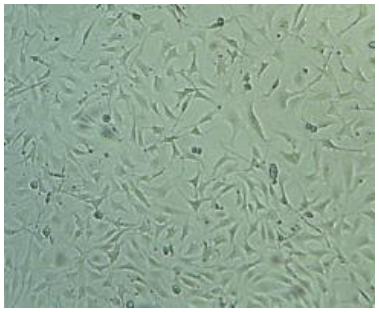
- Hydroxyl groups and compound configuration (aromatic rings): major determinants of antioxidant potential

# PME's effect on 3T3-L1 cell viability

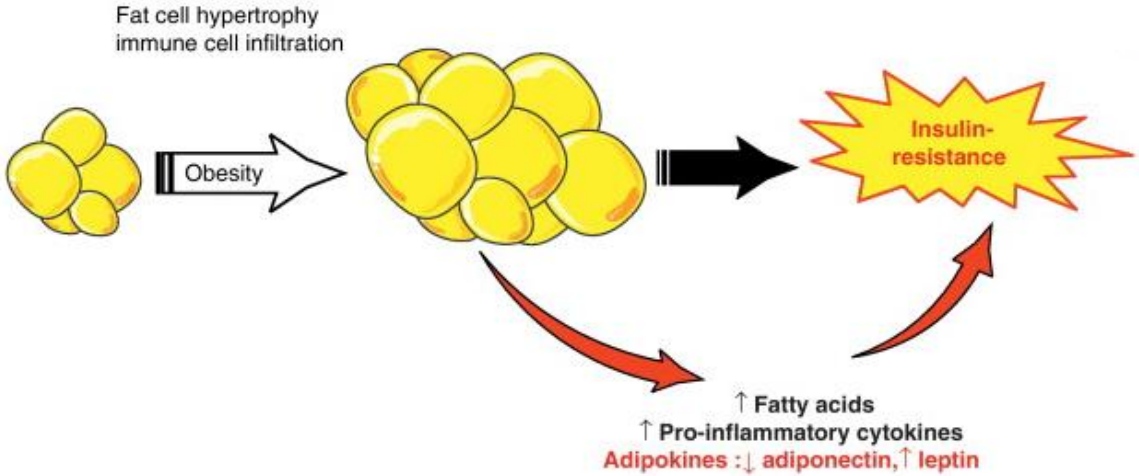
Freeze dried and ground. Exhaustively extracted by 70% methanol for 3 days. Lyophilised.

**Pomegranate extract (PME)**

Cell viability assays

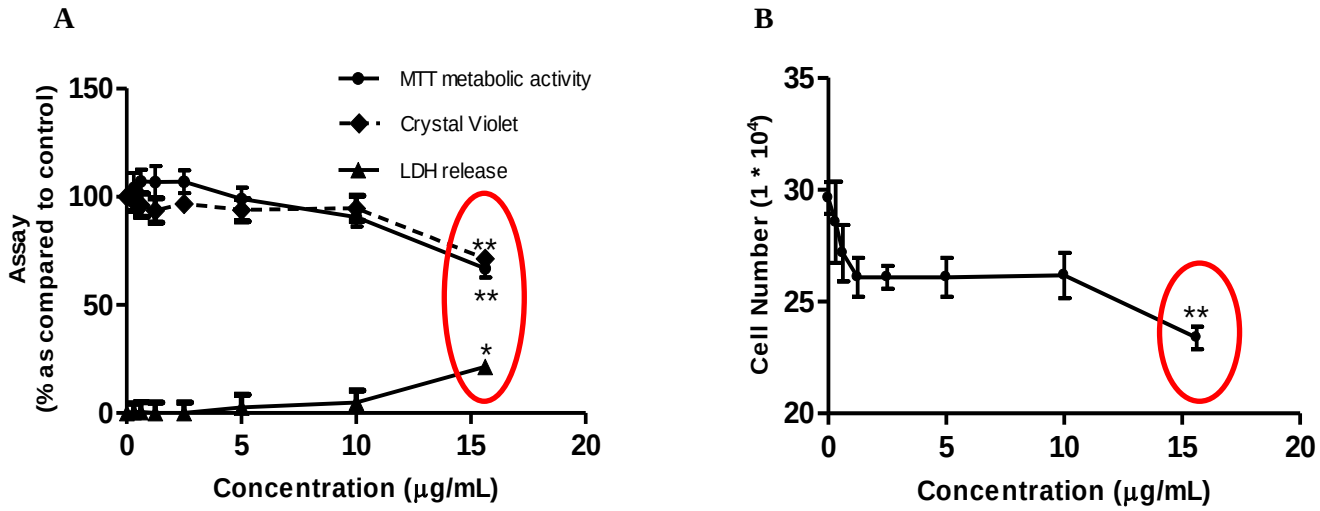


3T3-L1 preadipocytes: mimicking diabetes-like oxidative stress




(Castan-laurell *et al*., 2012)

# PME's effect on 3T3-L1 cell viability

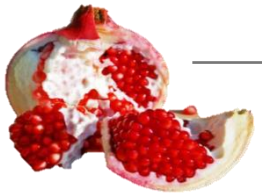



Gallic acid increased 3T3-L1 cell death in dose dependent manner (Hsu *et al.*, 2006)

Proportional conc. of flavonoids in lime juice induced apoptosis of human pancreatic cells (Patil *et al.*, 2009)

**Effect of PME on preadipocyte viability. Cytotoxicity assessed by (A) MTT metabolic activity, crystal violet and LDH release and (B) Trypan Blue exclusion methods.**

# PME's protective effect against oxidative stress



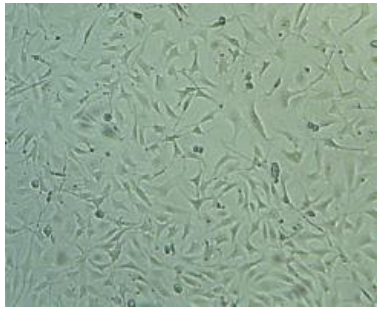
Freeze dried and ground. Exhaustively extracted by 70% methanol for 3 days. Lyophilised.

**Pomegranate extract (PME)**

Pretreatment with PME for 24h

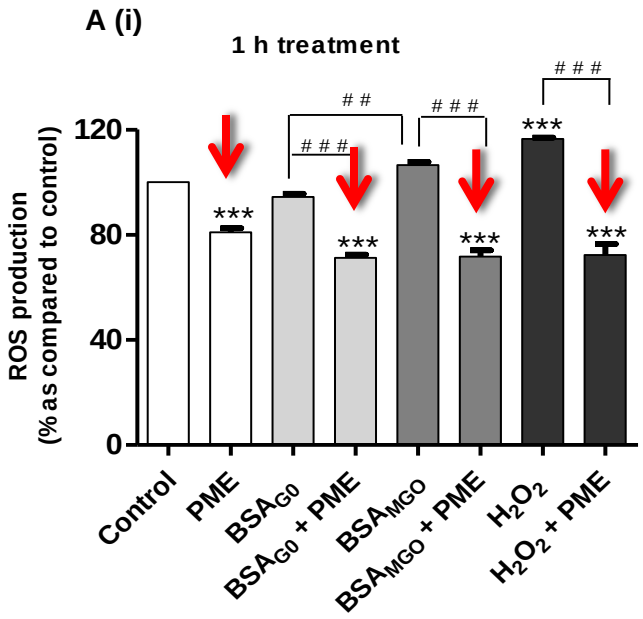
BSA + MGO => $BSA_{MGO}$ (AGEs)

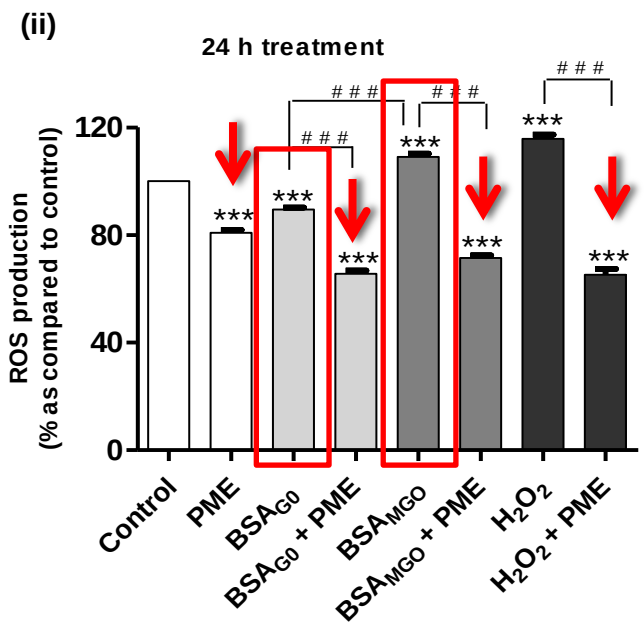
$H_2O_2$

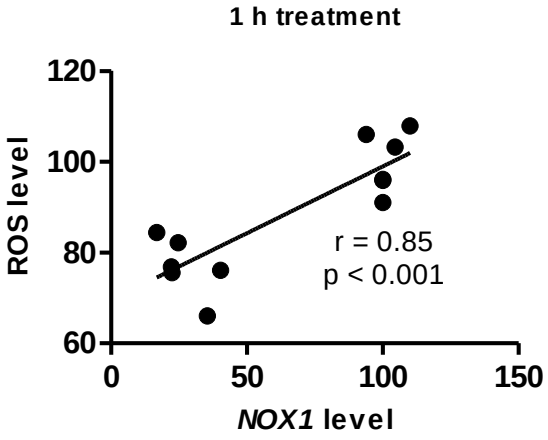


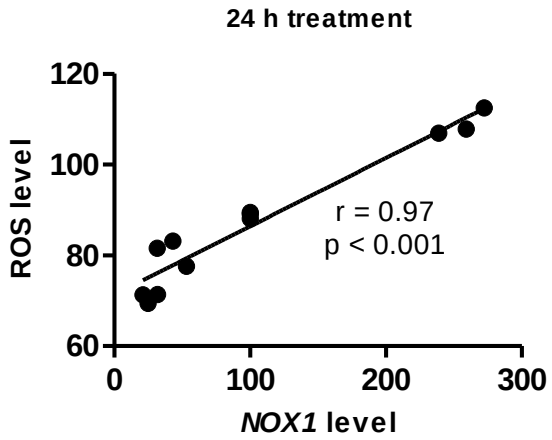
3T3-L1 preadipocytes: mimicking diabetes-like oxidative stress

# PME decreases ROS production



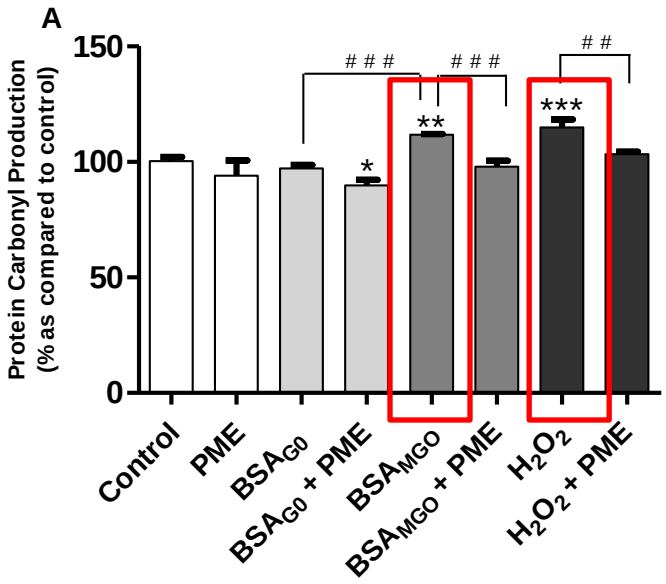



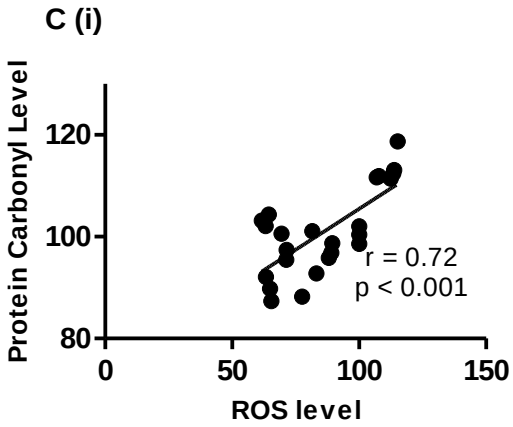



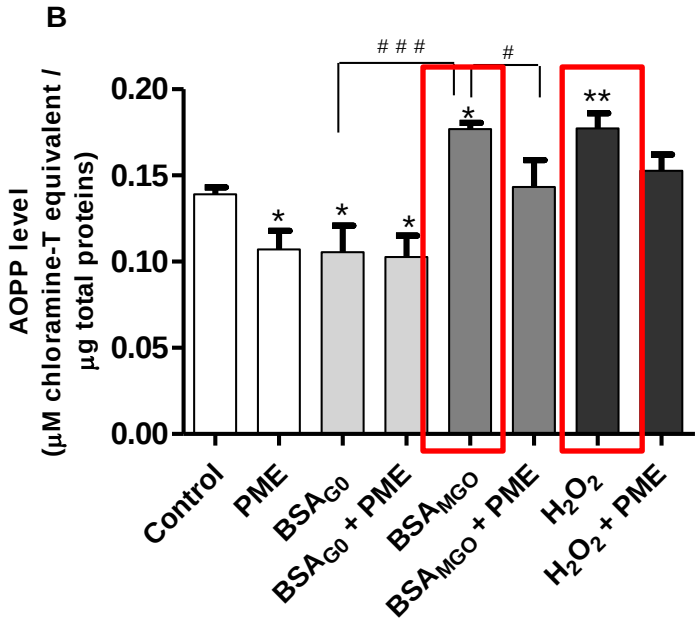



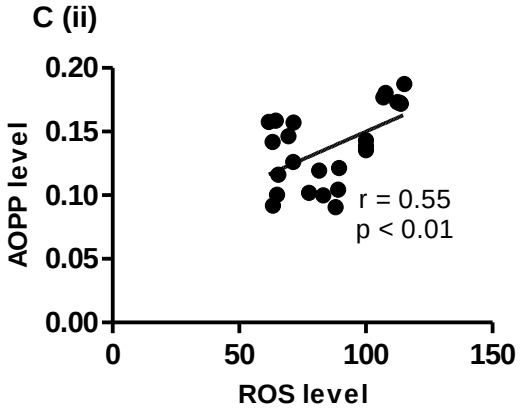

**Effect of PME on (A) intracellular ROS production at (i) 1 h and (ii) 24 h treatment; (B) mRNA expression *NOX1*.**

# PME reduces oxidatively modified proteins



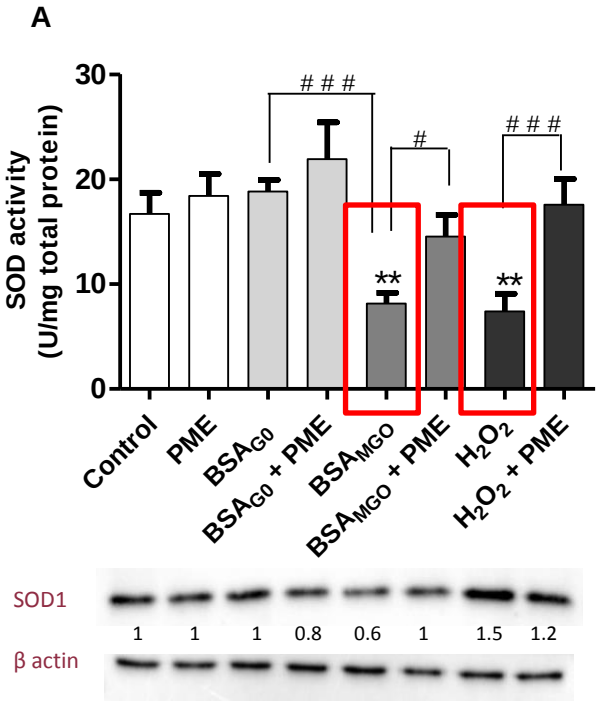



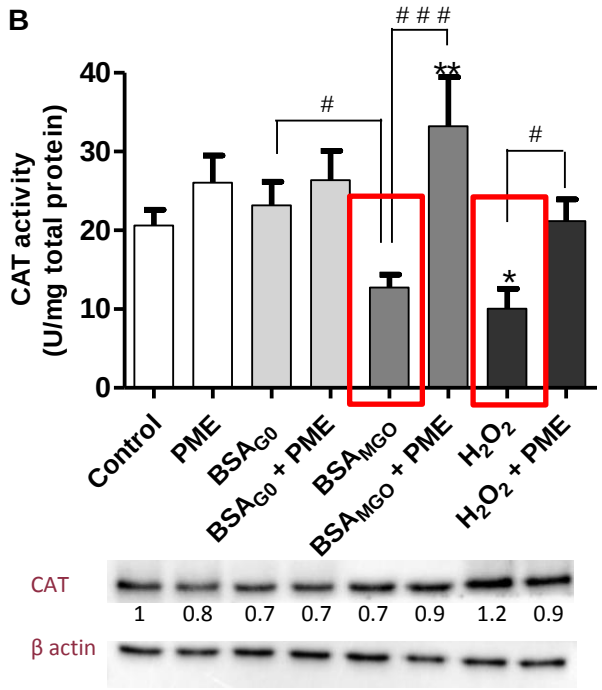



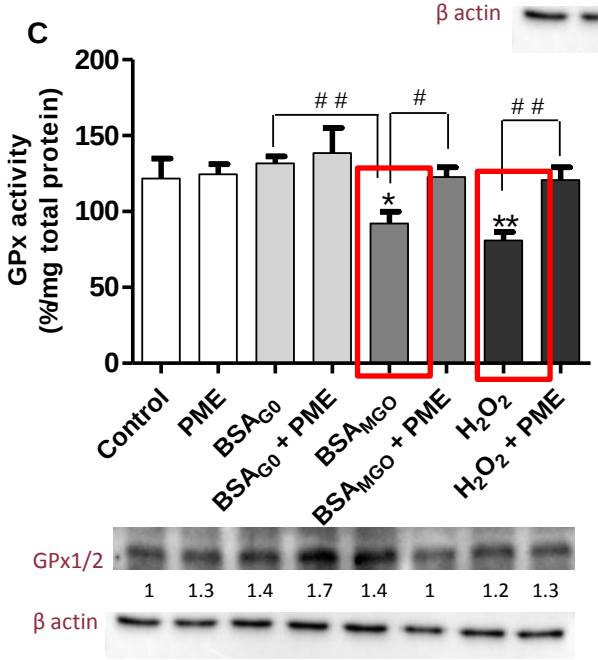




**Effect of PME on (A) protein carbonyls, (B) AOPP accumulations and (c) linear regression plots and Pearson's correlation coefficients between ROS level and (i) protein carbonyl and (ii) AOPP levels**

# Intrinsic antioxidant enzymes: expression & activity

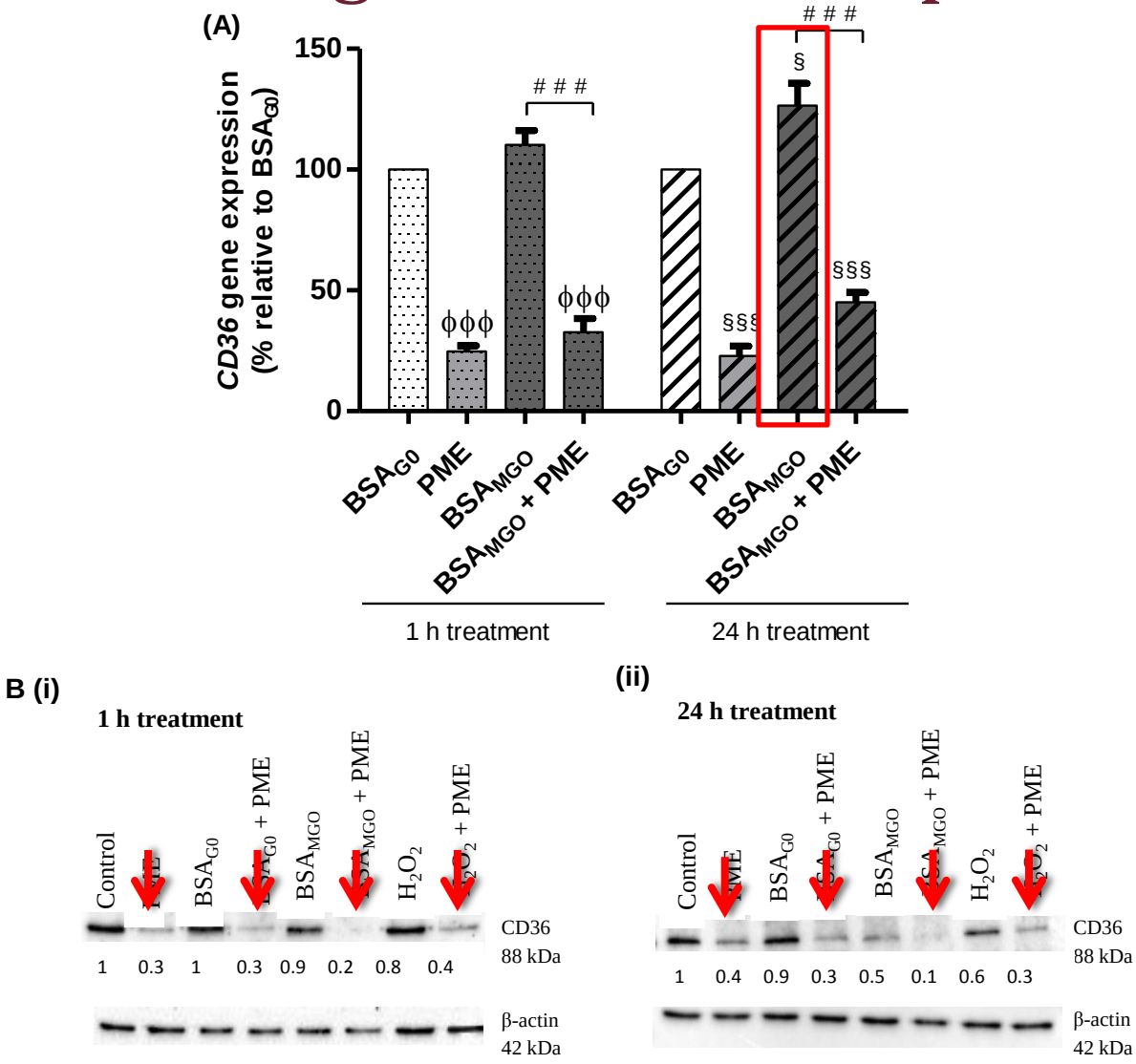


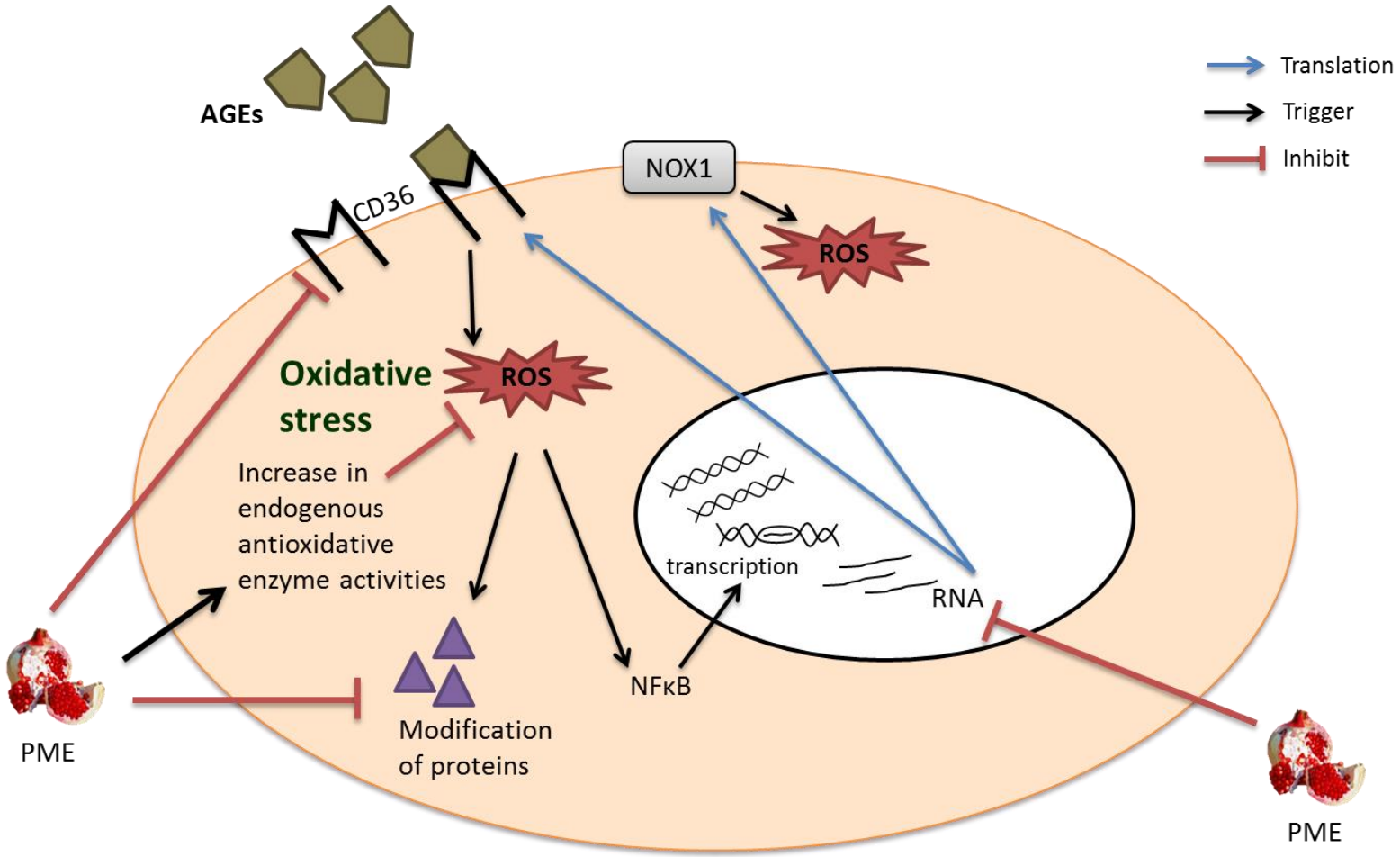

**Effect of PME (A) SOD, (B) CAT and (C) GPx enzymatic activities and expressions at 24h treatment.**
(Densitometry values are expressed relative to control and normalized against β-actin.

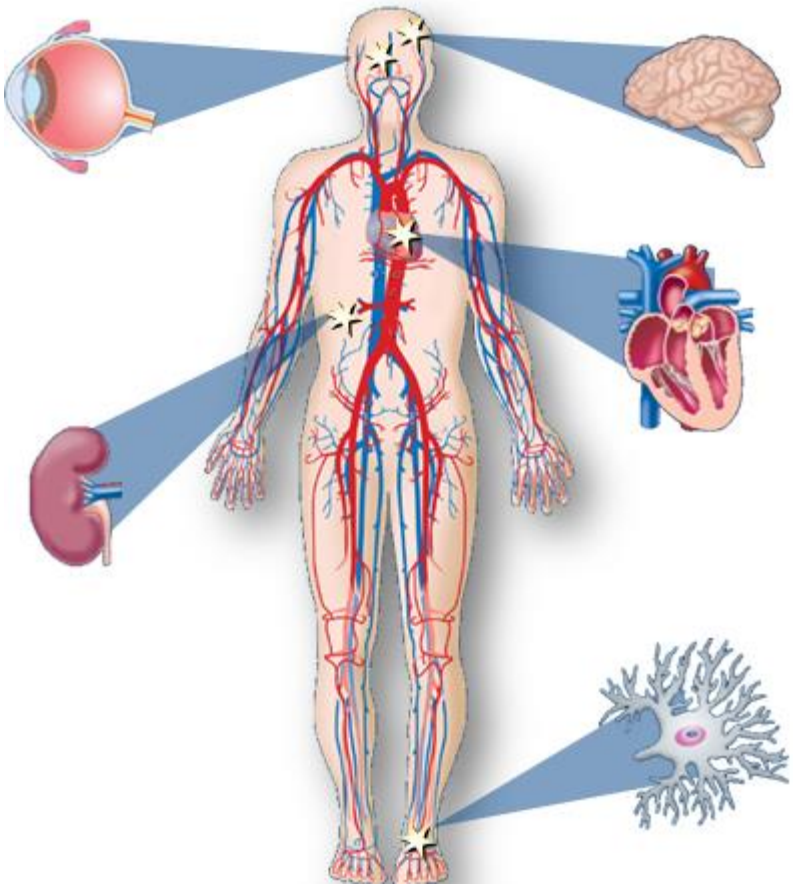
# PME down-regulates CD36 expression




**Effect of PME on (A) mRNA expression of *CD36* at 1 h and 24 h; (B) CD36 protein expression at (i) 1h and (ii) 24h**(Densitometry values are expressed relative to control and normalized against β-actin.

# Conclusion

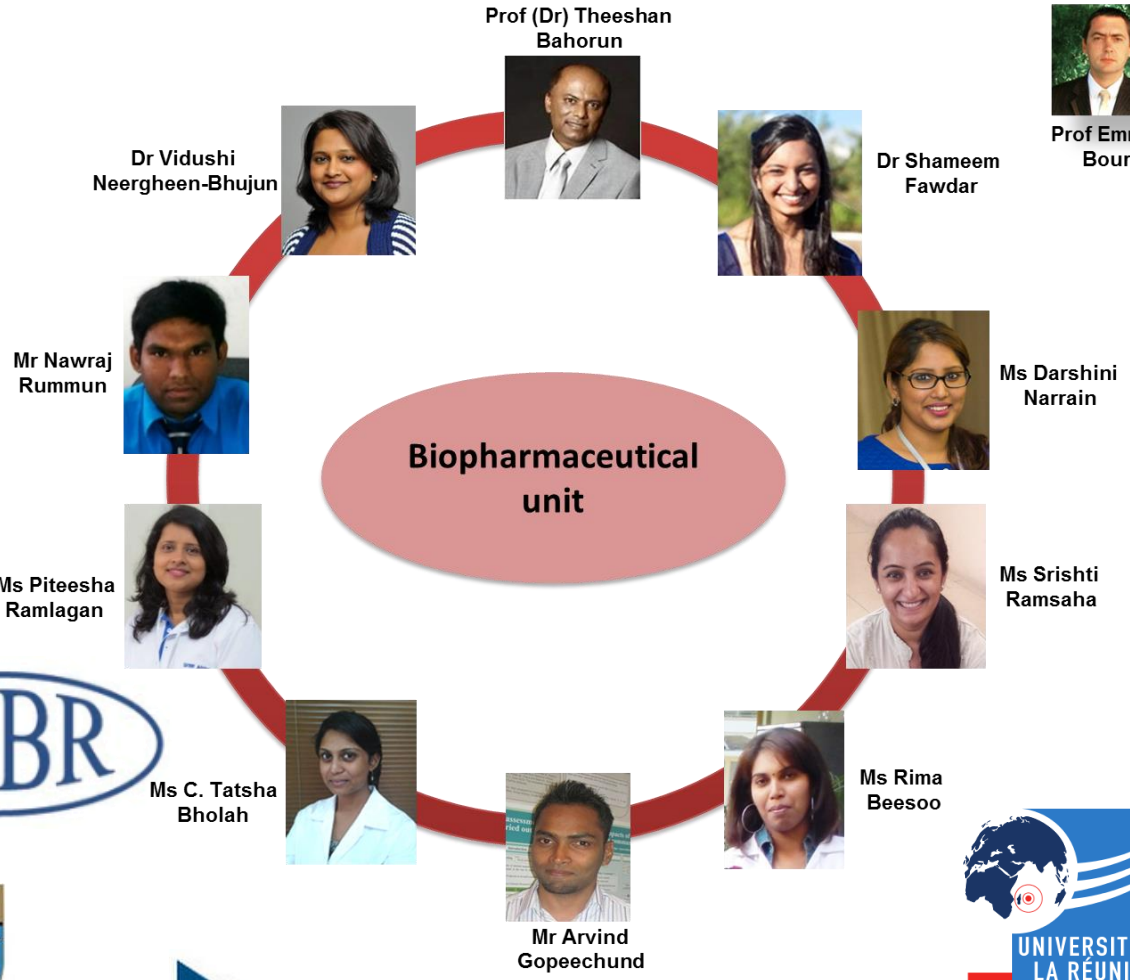

AGEs
CD36
NOX1
ROS
ROS
Oxidative stress
Increase in endogenous antioxidative enzyme activities
transcription
RNA
NFκB
Modification of proteins
PME
PME
Translation
Trigger
Inhibit



- PME: Propensity to mitigate obesity-related disorders

| Diabetes estimates (20-79 years) | Mauritius | Reunion | Madagascar | Comoros | South Africa |
|---|---|---|---|---|---|
| % of diabetic population | 18.3 | 13.0 | 1.5 | 3.6 | 4.2 |
| Mean diabetes related expenditure (USD), per person | 934.3 | NA | 111.4 | 152.2 | 1736.1 |

# Acknowledgement





Prof Emmanuel Bourdon


Dr Philippe Rondeau

DéTROI
Diabète - athérothrombose Thérapies Réunion Océan Indien

CBBR

UNIVERSITY OF MAURITIUS

MRC

QualiREG
La qualité pour le développement en Océan Indien

CYROI
Recherche Santé Bio-innovation

UNIVERSITÉ DE LA RÉUNION
S'ouvrir aux mondes

Liberté • Égalité • Fraternité
RÉPUBLIQUE FRANÇAISE

MINISTÈRE DE L'ENSEIGNEMENT SUPÉRIEUR ET DE LA RECHERCHE

# Thank you