The modulatory effect of pomegranate mesocarp on ribose-glycated protein

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**Introduction: Ribose**

- DNA/RNA backbone
- ATP synthesis

**Ribose**

- Abundant in the blood
- Contributes to HbA1c
- Potential biomarker for diabetes

Ribose is a sugar alcohol that is an essential component of DNA and RNA, involved in ATP synthesis and plays a role in the regulation of glucose metabolism, with potential applications in the monitoring of diabetes.
Estimated number of people with diabetes worldwide

(IDF Diabetes Atlas, 2017)
<table>
<thead>
<tr>
<th>Diabetes estimates (20-79 years)</th>
<th>Mauritius</th>
<th>Reunion</th>
<th>Madagascar</th>
<th>Comoros</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevalence, %</td>
<td>22</td>
<td>13.8</td>
<td>3.9</td>
<td>11.9</td>
</tr>
<tr>
<td>% of diabetic population</td>
<td>17.9</td>
<td>12.9</td>
<td>1.45</td>
<td>3.83</td>
</tr>
<tr>
<td>Diabetes related deaths</td>
<td>2609.3</td>
<td>NA</td>
<td>4685.6</td>
<td>339.7</td>
</tr>
<tr>
<td>Mean diabetes related expenditure (USD), per person</td>
<td><strong>535</strong></td>
<td>NA</td>
<td>27</td>
<td>100</td>
</tr>
</tbody>
</table>
Introduction: glycation

- Glucose
- Proteins

Schiff base
Amadori product

Advanced Glycation End products (AGEs)
Introduction: AGEs & diabetic complications

AGE receptor: CD36

Over-expressed by AGEs
Insulin resistance
Pro-atherogenic

AGE

AGE receptor

AGE receptor: CD36

Oxidative stress

ROS

Target genes

transcription

Pro-inflammatory cytokines

Vascular adhesion molecules

Diabetic complications
Functional food with anti-diabetic potential

Non-edible parts bioactive

Polyphenolic richness
Antioxidant potential
Suppresses CD36 expression
Anti-inflammatory capacities
Our focus

Glucose Proteins

Ribose
Glucose
Proteins

Schiff base
Amadori product

Ribose glycated
AGEs

AGE receptor
Oxidative stress

ROS
Inflammation
In vitro diabetic model

Anti-glycation assays
- Fluorescent AGEs
- Fructosamine
- Protein carbonyl
- AOPP

Pomegranate mesocarp extract (PME)

Cell viability
- ROS
- Protein carbonyl
- IL-6
Anti-glycative activity of PME

- Bovine serum albumin (BSA)
- Glucose/ribose
- PME/aminoguanidine

Incubation at 37 °C for 15 days

Glycation products

- Fluorescent AGEs
- Fructosamine
- Modified proteins
## Anti-glycative activity of PME

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Fluorescent AGEs (% BSA)</th>
<th>Fructosamine (mM DDMF)</th>
<th>Protein carbonyl (BSA) (%)</th>
<th>AOPP (nmol chloramine-T/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSA</td>
<td>100</td>
<td>0.711 ± 0.10</td>
<td>100</td>
<td>0.07 ± 0.01</td>
</tr>
<tr>
<td>BSA + GLU+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMSO</td>
<td>176.21 ± 2.32***</td>
<td>4.97 ± 0.28***</td>
<td>107.29 ± 5.13</td>
<td>0.33 ± 0.05</td>
</tr>
<tr>
<td>PME</td>
<td>102.15 ± 0.49 ###</td>
<td>2.99 ± 0.29###</td>
<td>100.26 ± 11.62</td>
<td>0.35 ± 0.08</td>
</tr>
<tr>
<td>Aminoguanidine</td>
<td>103.46 ± 0.60 ###</td>
<td>3.49 ± 0.26***</td>
<td>94.61 ± 5.21</td>
<td>0.36 ± 0.03</td>
</tr>
<tr>
<td>BSA + RIB+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMSO</td>
<td>1346.69 ± 20.82***</td>
<td>4.86 ± 0.15***</td>
<td>175.01 ± 6.14***</td>
<td>3.91 ± 0.18***</td>
</tr>
<tr>
<td>PME</td>
<td>103.36 ± 12.36 ###</td>
<td>3.14 ± 0.35###</td>
<td>99.82 ± 9.23</td>
<td>1.48 ± 0.27###</td>
</tr>
<tr>
<td>Aminoguanidine</td>
<td>114.85 ± 18.10 ###</td>
<td>3.12 ± 0.25###</td>
<td>131.85 ± 5.94*##</td>
<td>2.55 ± 0.14###</td>
</tr>
</tbody>
</table>
Effect of PME in *in vitro* diabetic model

Pretreatment with PME for 24h

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

BSA

BSA$_{\text{RIB}}$ (AGEs)
PME protects against AGEs-induced cell death

MTT cell viability (% of control)

- Control
- BSA$_{G0}$
- BSA$_{RIB}$

LDH leakage (% of control)

- Control
- BSA$_{G0}$
- BSA$_{RIB}$
PME counteracts AGEs-induced oxidative stress and inflammation

**ROSA production (% of control)**
- Control
- PME

**IL-6 secretion (pg/mL)**
- Control
- PME

Graphs show a comparison between Control and PME for ROS production and IL-6 secretion under different conditions.
PME lowers accumulation of oxidatively modified proteins

Protein carbonyl level (% of control)

- Control
- BSA<sub>G0</sub>
- BSA<sub>RIB</sub>

AOPP level (nmol chloramine-T equivalent/mg total proteins)

- Control
- BSA<sub>G0</sub>
- BSA<sub>RIB</sub>

** Protein carbonyl level
- Control: 100
- BSA<sub>G0</sub>: 110
- BSA<sub>RIB</sub>: 130

** AOPP level
- Control: 10
- BSA<sub>G0</sub>: 15
- BSA<sub>RIB</sub>: 20

r = 0.87
p < 0.001

r = 0.73
p < 0.001
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

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Total phenolic content</th>
<th>Total flavonoid content</th>
<th>Hydrolysable tannin content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude</td>
<td>483.97 ± 18.32(^b)</td>
<td>383.78 ± 9.20(^c)</td>
<td>704.52 ± 7.74(^d)</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>80.48 ± 2.85(^e)</td>
<td>18.04 ± 0.34(^e)</td>
<td>127.61 ± 7.09(^e)</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>570.14 ± 14.13(^a)</td>
<td>533.85 ± 12.02(^b)</td>
<td>901.46 ± 11.64(^b)</td>
</tr>
<tr>
<td>n-butanol</td>
<td>323.27 ± 6.40(^d)</td>
<td>681.75 ± 5.97(^a)</td>
<td>945.69 ± 12.86(^a)</td>
</tr>
<tr>
<td>Water residue</td>
<td>431.78 ± 8.01(^c)</td>
<td>350.25 ± 14.71(^d)</td>
<td>739.08 ± 3.89(^c)</td>
</tr>
</tbody>
</table>
# Antioxidant activity of pomegranate mesocarp fractions

<table>
<thead>
<tr>
<th>Fraction</th>
<th>IC$_{50}$ value (µg/mL) for</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ABTS radical scavenging</td>
</tr>
<tr>
<td>Crude</td>
<td>2.340 ± 0.017$^a$</td>
</tr>
<tr>
<td>Dichloro-methane</td>
<td>39.732 ± 1.752$^b$</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>1.638 ± 0.081$^a$</td>
</tr>
<tr>
<td>n-butanol</td>
<td>1.181 ± 0.042$^a$</td>
</tr>
<tr>
<td>Water residue</td>
<td>2.574 ± 0.177$^a$</td>
</tr>
</tbody>
</table>
**Pomegranate mesocarp fractions modulate CD36 expression**

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.

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

AGEs

ROS

Modified proteins

Transcription

IL-6

Inhibit

Intermediate products

Glucose

Ribose

Proteins

PME

Nucleus

Trigger
Acknowledgement
Thank you
Merci
Misaotra anao
Marahaba