



## Role of *Vigna Radiata* extracts in modulating oxidative stress in an *in vitro* cell system

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*Vigna Radiata*, or mung bean, is commonly consumed in Asia and, in recent years, has become increasingly popular in western countries as part of a healthy dietary pattern. Its content of balanced nutrients, including protein and dietary fibres, and significant amounts of bioactive phytochemicals are thought to be the main contributors to its anti-tumour and anti-inflammatory activity, to lipid metabolism regulation and, more generally, to its health properties<sup>(1)</sup>. Sprouts of mung bean after germination have been shown to have increased biological activities and to improve the nutritional and medicinal qualities of *Vigna Radiata*<sup>(2)</sup>. However, the ability of *Vigna Radiata* and its sprouts to counteract oxidative stress (OS), typically associated with obesity, has not been previously assessed. Obesity is characterised by systemic OS, deemed to play a key role in the development of co-morbidities such as cardiovascular disease, type 2 diabetes and certain type of cancer. Moreover, obesity is associated with activation of monocytes and increased expression of a variety of cytokines contributing to increased proinflammatory markers and reactive oxygen species (ROS) concentrations in the obese<sup>(3)</sup>. This study aims, therefore, to assess the anti-oxidant properties of extracts from raw and germinated *Vigna Radiata* in an *in vitro* cell system for monocytes and colon cancer.

Raw and germinated *Vigna radiata* flours were extracted twice at pH 2 for 45 min with a mixture of acetone:water:HCl (70:30:0.1) using a ratio of legume flour/extracting solution of 40 mg/mL. Evaporated extracts were reconstituted in ethanol (0.1%) to a final concentration of 8.9 mg/mL. Polyphenol content in both extracts was quantified using the Folin-Ciocalteu method. U937 monocyte cells and HT-29 colon cancer cells were cultured for 24 hours at 37 °C before being supplemented, or not, with extract from raw (VRR) and 3 day germinated (VRG3) *Vigna Radiata* (0.89 mg/mL) and treated with paraquat (1 mM) (PQ) and S-Nitroso-N-acetyl-DL-penicillamine (0.7 mM) (SNAP) overnight to induce OS. Cell viability was assessed *via* MTS or MTT assay; ROS generation was determined by Flow Cytometry using CM-H<sub>2</sub>DC-FDA while antioxidant GPx1 and GPx4 enzymes gene expression was quantified by semi-quantitative RT-PCR. Three independent experiments were performed for each cell line and, in each experiment, at least 3 replicates for each condition tested were included. Results are expressed as a percentage of untreated control cells, equal to 100%.

PQ/SNAP treatment significantly reduced U937 cell viability by 75% and significantly increased ROS generation by 8 fold, compared to untreated control U937 cells ( $p < 0.05$ ); confirming the induction of OS. Addition of 0.89 mg/mL VRR and VRG3 extracts significantly increased U937 cell viability by 37 and 33%, respectively ( $p < 0.05$ ), when compared to PQ/SNAP treated cells. Furthermore, VRR and VRG3 extracts supplementation significantly reduced ROS generation by 9 and 19% ( $p < 0.05$ ), respectively, in U937 cells treated with PQ/SNAP. GPx1 and GPx4 gene expression was negatively affected by the addition of VRR and VRG3 extract and further affected by PQ/SNAP treatment. A similar but greater effect of both extracts was observed in HT-29 colon cancer cells: cell viability was increased by 174 and 135% after incubation with VRR and VRG3 respectively ( $p < 0.05$ ) when compared to PQ/SNAP treated cells.

Results obtained in this study suggest that extracts from *Vigna Radiata* and its sprouts may be effective in reducing ROS generation and improving cell viability. However the involvement of the antioxidant genes tested is not clear. VRG3 extracts have been shown to be more effective possibly due to the higher content in polyphenols (386 vs 332 µg/mL). These preliminary data support the potential use of *Vigna Radiata* and its sprouts in the diet as a source of antioxidant compounds to reduce systemic OS and prevent co-morbidities in overweight/obese individuals.

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