Protective Effect of Coconut Cake Phenolic Antioxidants on Oxidative Stress Induced Macromolecular Damage in HEp-2 Cells

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Coconut cake, a by-product of the coconut oil manufacturing is a rich source of phenolic antioxidants. The majority of research dealing with phenolic antioxidants is primarily focused on the extraction of phenolic substances from plant materials and assessment of antioxidant properties in chemical systems. However, such assays in chemical systems do not guarantee the antioxidant properties of phenolic substances in biological systems. In this study, inhibition of H₂O₂ induced oxidative damage on lipids and proteins by coconut cake phenolic antioxidants (CCPA) was studied in HEp-2 cells as the biological system. CCPA were extracted with 70 % ethanol and the total polyphenol content was measured by Folin Ciocalteu method. The CCPA content, calculated as gallic acid equivalents was 182.81 ± 28.73 mg/kg. The o-diphenols content, calculated as caffeic acid equivalent using a method reported by Gutfinger was 66.83 ± 16.50 mg/kg. Oxidative damage in HEp-2 cells was induced by adding H₂O₂in PBS for 1 hr. The maximum concentration of H₂O₂ that does not affect the cell viability (>99 %) was determined as 100 µM using Cell-Titer Glo Luminescent Cell Viability Assay. Formation of thiobarbituric acid reactive species (TBARS) due to lipid peroxidationin HEp-2 cells (0.010±0.000 µM/mL) compared to the control (0.007±0.000 μM/mL) without H₂O₂was inhibited with 0.5mg/mLCCPA (0.007±0.000μM/mL). Protein oxidation (3.05±0.06nmol/mL) compared to the control (2.14±0.06nmol/mL) without H₂O₂ as assessed by protein carbonyl formation assay with 2, 4-dinitophenylhydrazine was also inhibited by treating the HEp-2 cells with 0.5mg/mL CCPA (2.41±0.06 nmol/mL). Thus, CCPA caninhibit oxidative stress-induced macromolecular damage of lipids and proteins in biological systems.

Keywords: Coconut Cake Phenolic Antioxidant, Oxidative stress, MDA, Protein oxidation

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