Protective effect of coconut oil meal phenolic antioxidants on mitochondrial DNA damage in HEp-2 human epithelial cells

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Coconut oil meal is a rich source of phenolic antioxidants. In most of the reported research, antioxidant activities of phenolic extracts have been tested using chemical systems. However, similar studies conducted in biological systems is relatively less common. In this study, glutathione oxidation and mitochondrial DNA (mt-DNA) damage in HEp-2 cells were used as biological reactions to assess protective effect of coconut oil meal phenolic antioxidants (CMPA). CMPA were extracted with ethanol water solvent system (70% v/v) and the total phenolic content was measured by Folin Ciocalteu method. HEp-2 cells at 80% confluence were treated with 0.5 mg/ml CMPA overnight. Oxidative stress in HEp-2 cells was induced by exposing cells to H_2O_2 (10, 50, 100, 250 and 500 µM) for 1hr. The maximum concentration of H_2O_2 that does not affect the cell viability (>99%) was 100 µM as measured by Cell-Titer Glo Luminescent Cell viability assay (Promega). Glutathione (GSH) to oxidized glutathione (GSSG) ratio (GSH/GSSG) was measured by GSH/GSSG Glo assay (Promega). To evaluate the mt-DNA damage, total DNA of high purity (A_{260nm}/ A_{280nm} >1.8) was extracted from stressed and unstressed cells pretreated with CMPA using HiPurA™ Blood Genomic DNA Miniprep Purification Kit. Two primer sets that amplify a short and long fragment of mt-DNA in the D-loop region were selected to assess damage in the D-loop region that is known to be more prone to DNA damage. Quantitative Real time PCR was carried using the QuantiTect SYBR Green PCR kit (Qiagen) according to the manufacturer's instructions and the amplification was monitored with StepOne quantitative thermal cycler (Applied Bio). The ratio of intact DNA was calculated according to the following equation; Lesion rate [Lesions per 10kb DNA] = $(1-2^{-(\Delta \text{long} - \Delta \text{short})})$ x (10000 bp/Size of long fragment bp). Total CMPA was 1892 ± 51 mg/kg as gallic acid equivalents .Two sample t-test was carried out for the determination of significant differences ($p \leq 0.05$) between the mean values .The GSH/GSSG ratio of the samples pretreated with CMPA, subjected to H2O2 oxidative stress (100 µM) (5.01±0.08 (was significantly higher)P<0.05 (compared to that of samples subjected to H2O2 oxidative stress without pretreatment)2.19±0.04) while GSH/GSSG ratio of control cells without any treatment was 5.19±0.20. In mt-DNA damage assay, the samples pretreated with CMPA, subjected to H_2O_2 oxidative stress (100 µM) showed significantly less (P<0.05) lesions (7.65±0.06 lesions/10 kb DNA) compared to samples subjected to H_2O_2 oxidative stress without pretreatment (9.38±0.60 lesions/10 kb DNA). Thus, CMPA can inhibit glutathione oxidation and mt-DNA damage in HEp-2 cells suggesting that CMPA may help reduce health conditions associated with oxidative stress.

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