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INHIBITION OF ADVANCED GLYCATION END PRODUCTS (AGE) FORMATION BY POMEGRANATE (PUNICA GRANATUM L.) STEM BARK METHANOL EXTRACT

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Advanced glycation end products (AGEs), aregenerated through non-enzymatic binding of glucose to free amino groups of an amino acid. Carboxymethyllysine (CML), as a non-fluorescent AGE, is recognized as a biomarker of formation AGEs compounds. Accumulation of AGEs in tissues promotes diabetic complications. Various parts of pomegranate tree possess polyphenol antioxidants. The study was conducted to examine the inhibitory potential of crude extract of pomegranate stem bark on formation of CML. Therefore, The glycation of bovine serum albumin (BSA) was carried out according to the usual method (Peng et al., 2008). BSA (20 mg/ml) was dissolved in phosphate buffer saline (pH=7.4) and incubated at 37 ^{o}C in the presence of D-glucose (5.5 or 250 mM) with/without different concentrations of methanol extract of pomegranate stem bark as 85, 170 and 250 µg/ml. Aminoguanidine (AG) 1mM was used as positive control. In addition, the final solution of the reaction mixtures received 0.2 g/L sodium azide (NaN3) to assure an aseptic condition. The reaction mixtures were incubated at 37°C for 30 days. The Effect of extract and positive control on formation of advanced glycation end products was assessed by competitive ELISA Kit (Catalog number STA-817). The AGE-BSA adduct was quantified in the samples by comparison of their absorbance with that of a known AGE-BSA standard curve. The results demonstrated compared to control extract inhibited formation of CML in BSA-glucose system in concentration dependent manner. Once BSA was incubated with glucose$_{5.5}$ mM, the extract in concentrations of 85, 170, and 250µg/ml reduced AGE formation by 43.66%, 69.58%, and 77.66 respectively compared to BSA-Glu$_{5.5}$ as control. All mixture reactions containing samples showed markedly difference with BSA-Glu$_{5.5}$ as a control. In diabetic model (BSA+Glu$_{250}$) experiment, the results revealed that extract reduced AGE formation in concentration dependent manner. Extract in concentration of 250µg/ml with 77.51% reduction in CML formation had no statistically differences with aminoguanidine as positive control.

References

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