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GEL ELECTROPHORESIS - A TOOL TO ANALYZE *IN-VITRO* PROTEIN GLYCATION AND INHIBITION

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Protein glycation is a non-enzymatic reaction which occurs between reducing sugars and free amino groups of proteins which is a major modification that compromises the function of the affected protein. Advanced glycation end products have been implicated in the pathogenesis of age-related chronic diseases and micro and macrovascular complications associated with diabetes. Several expensive analytical techniques requiring special equipment have been developed in an attempt to identify natural protein glycation inhibitors. The objective of this study was to develop a simple electrophoresis-based method to detect and analyze acceleration and inhibition of *in-vitro* protein glycation which will be used later to screen medicinal plants for their effects on protein glycation.

Bovine Serum Albumin (BSA) was incubated at 37°C in phosphate buffer at pH 7.4 under sterile conditions, with varying concentrations of glucose, fructose and ribose in the presence or absence of aminoguanidine (AG) a known standard glycation inhibitor. Appropriate controls were used. Aliquots of the incubated samples were collected at intervals up to 30 days and analyzed using polyacrylamide gel electrophoresis (PAGE) under non-denaturating conditions. Changes in the migration position of the BSA bands were analyzed in non-glycated, glycated and inhibited reactions after staining the gel.

Migration of the BSA band showed clearly detectable variation under the experimental conditions used. BSA migration was increased with increasing sugar concentration and the duration of incubation. AG showed a dose-dependent inhibition of BSA migration, in the presence of sugar. Three sugars used showed a variation in the rate of protein modification during early incubation, where ribose showed the most rapid effect while glucose showed the slowest. However, with longer incubations the rate of modifications looked relatively similar with the sugars used.

We demonstrated many aspects of protein glycation using a comparatively simpler method, which were previously demonstrated by using more sophisticated and expensive equipment. Positively charged free amino groups are targets of glycation, to which negatively charged reducing sugars are attached. Variation in the albumin migration seems to be proportionate to the number of cationic residues modified by glycation. We reveal the capability of the technique developed, in screening potential inhibitors of protein glycation, indicating this method as a useful tool in the discovery of new natural therapeutics for diabetes and age-related chronic diseases.

In conclusion, we have developed a method which can detect and analyze *in-vitro* acceleration and inhibition of protein glycation using PAGE. Further studies are in progress to identify natural inhibitors of protein glycation using the method developed.

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