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A STUDY ON THE ROLE OF DIETARY AGENT *MOMORDICA CHARANTIA* L ON PROTEIN GLYCATION

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Momordica charantia L, AGEs-advanced glycation end products, HSA- human serum albumin.

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ABSTRACT: Protein glycation is a spontaneous post translational modification of proteins by excess sugars causing formation of advanced glycation end products (AGEs) in diabetic individuals and responsible for diabetes complications. A wide variety of anti-glycating agents have been reported & recently there has been interest in natural products with anti-glycation properties. *Momordica charantia* L (*M. Charantia* L) has been used historically for medicinal purposes particularly for treatment of diabetes & cancers. *M. Charantia* L Extract contains potent antioxidant activity and it can be a useful anti-glycating agent. **Material & methods:** Human serum albumin was used for in vitro glycation. Various concentrations of extract of *M. Charantia* L were analyzed. **Results:** Co-incubation of the *M. Charantia* L. extract with HSA-fructose mixture intensify the fructose mediated glycation of HSA as indicated by increases fluorescence intensity in tryptophan fluorescence & AGE related fluorescence studies. **Conclusion:** *M. Charantia* L. seems to aggravate sugar mediated glycation of the protein and need further studies to pinpoint specific bioactive compounds responsible for the observed activities.

INTRODUCTION: Glycation is a spontaneous post-translational modification of proteins in which reducing sugars bind covalently to the free amino groups of proteins which leads to formation of advanced glycation endproducts (AGEs)¹⁻². AGEs contribute to the onset of several diseases such as diabetic complications, renal insufficiency, and Alzheimer's disease³⁻⁴.

Recently, attention has been focused on preventing protein glycation by antioxidant from plant sources. Thus far, some compounds such as aminoguanidine, aspirin, Vitamin B6, taurine, quercetin and anti-inflammatory drugs including ibuprofen, are reported to be inhibitors of the glycation reaction⁵⁻¹⁰.

Momordica charantia L (Cucurbitaceae), commonly known as bitter gourd or karela, is a tropical vegetable. It is popularly used medicinal plant in Asia, India, Africa and South America for treating various diseases such as diabetes and cancers¹¹⁻¹³. As regards to its chemical composition, it has a non-nitrogenous neutral

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principle charantin and on hydrolysis gives glucose and a sterol. The fruit pulp of *M. Charantia* has soluble pectin but no free pectic acid. Galactouronic acid is also obtained from the pulp. *M. Charantia* fruit contains glycosides, saponins, alkaloids, reducing sugars, resins, phenolic constituents, fixed oil and free acids. The presence of an unidentified alkaloid and 5-hydroxytryptamine is also reported. The ether extract residue of the alcoholic concentrate from the leaves of *M. charantia* is reported to reveal hypoglycemic activity comparable to that of tolbutamide¹⁴. Hence, our study endeavors to analyze the effect of *M. Charantia* L extract on *in vitro* HSA (Human Serum Albumin) glycation produced by fructose, a more potent AGE forming agent than glucose to induce more AGE formation in the system. Several studies have shown that the chemical inhibition of HSA glycation helps attenuate diabetic complications. Hence, the study can be useful in providing insight for prevention of secondary complications of diabetes.

MATERIALS AND METHODS:

This study was undertaken in the Department of Biochemistry, J. N. Medical College, Aligarh Muslim University, Aligarh (Uttar Pradesh), India.

Materials:

Human serum albumin (HSA), dinitro phenyl hydrazine (DNPH), ethylene tetradiamine tetraacetic acid (EDTA), Coomassie brilliant Blue R-250, sodium dodecyl sulphate (SDS), agarose and dialysis membranes of one inch diameter were purchased from Sigma Chemical Company, U.S.A. The *M. Charantia* L. was purchased from a local market in Aligarh.

Equipments:

Digital pH meter, type DPH-100, Shimadzu RF-5301 PC Spectrofluorometer, Beckman-DU-640B Spectrophotometer, Lyophilizer - HETO, Photochem-8 colorimeter, Microplate reader-Qualisystem PR-601, Polyacrylamide gel electrophoresis assembly (Genei Bangalore) were the major equipments used in this study.

METHODS:

Isolation of Plasma albumin: Plasma albumin was isolated by the method of Tayyab & Qasim (1990)

¹⁵. Human blood was procured from the Emergency operation theatre (O.T.)/Blood Bank of Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh.

Determination of Protein Concentration:

Protein concentration was determined by the method of Lowry *et al.*, (1951) using bovine serum albumin as the standard¹⁶. In this method of protein estimation, two reagents, namely, Folin & Ciocalteu's phenol reagent and copper reagent were used.

Gel Chromatography:

Sephacryl S-100 HR column was used for gel chromatography.

Polyacrylamide Gel Electrophoresis:

Polyacrylamide gel electrophoresis of HSA was carried out in tris-glycine buffer, pH 8.3 on 7.5 % polyacrylamide gels according to the method of Laemmli (1970)¹⁷.

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis:

Sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis was performed by the tris-glycine buffer system of Laemmli (1970) using slab gel electrophoresis apparatus¹⁷.

Staining Procedures:

After the electrophoresis was complete the gels were removed and the protein bands were visualized by Coomassie brilliant blue staining.

Spectral Analysis:

Ultraviolet Absorption Spectroscopy:

The ultraviolet absorption spectra of native and glycated HSA samples were recorded in the wavelength range 200-400 nm on a Beckman-DU-640B spectrophotometer, using a cuvette of 1 cm pathlength. One mg of native and glycated HSA in a total volume of 3.0 ml was taken for spectral analysis.

Tryptophan Fluorescence:

The fluorescence of tryptophan residue Trp214 in native and glycated HSA was monitored with excitation at 285 nm and the emission measured

over the range 290-440 nm¹⁸. The concentration of protein samples was taken as 100 µM.

Advanced Glycation End Products (Ages) Related Fluorescence:

AGEs formations were measured by determining the fluorescence by excitation at 370 nm and emission between 400-500nm using Shimadzu RF-5301 PC spectrofluorophotometer¹⁹. The concentration of protein sample was taken as 100 µM.

Determination of Protein Bound Carbonyl Groups:

HSA bound carbonyl groups were estimated by a published procedure²⁰.

Determination of Total Phenolic Content:

Total phenolic content was determined by the method of Saucier *et al.* (1999) with slight modification and the results were expressed directly in absorbance units at 765 nm²¹.

RESULTS:

Total Polyphenolic Compounds:

The extract of *M. Charantia* L was found to have good amount (75.3 mg/g) of polyphenolic compounds.

Tryptophan Fluorescence:

Glycated HSA showed decrease in fluorescent intensity as can be seen from **Fig. 1**, glycated HSA samples treated with *M. Charantia* L extract showed decrease in fluorescence intensity in a dose-dependent manner.

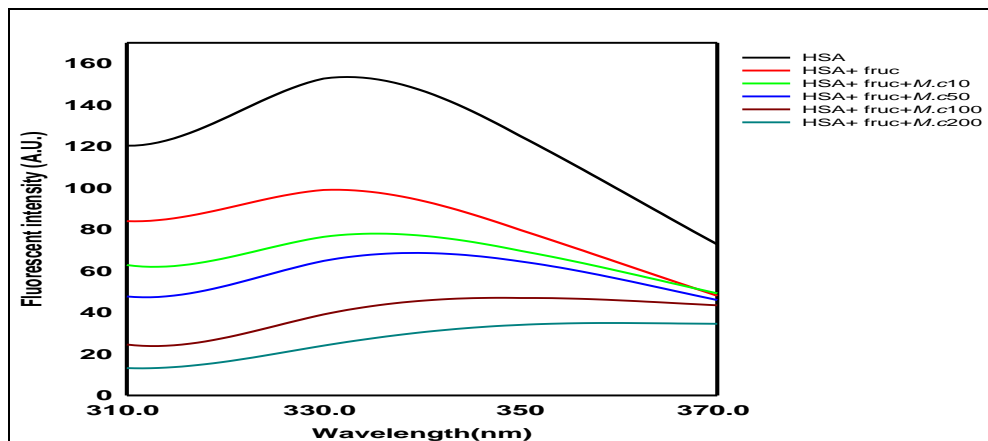


FIG.1: TRYPTOPHAN FLUORESCENCE OF HAS UPON *IN VITRO* GLYCATION IN THE PRESENCE AND ABSENCE OF *M.CHARANTIA* L

AGE-Specific Fluorescence:

AGE-specific fluorescence was found to increase in the presence of fructose following the 21-days

incubation. Presence of extract of *M. Charantia* L led to further increase in formation of fluorescent AGEs in a dose dependent manner.

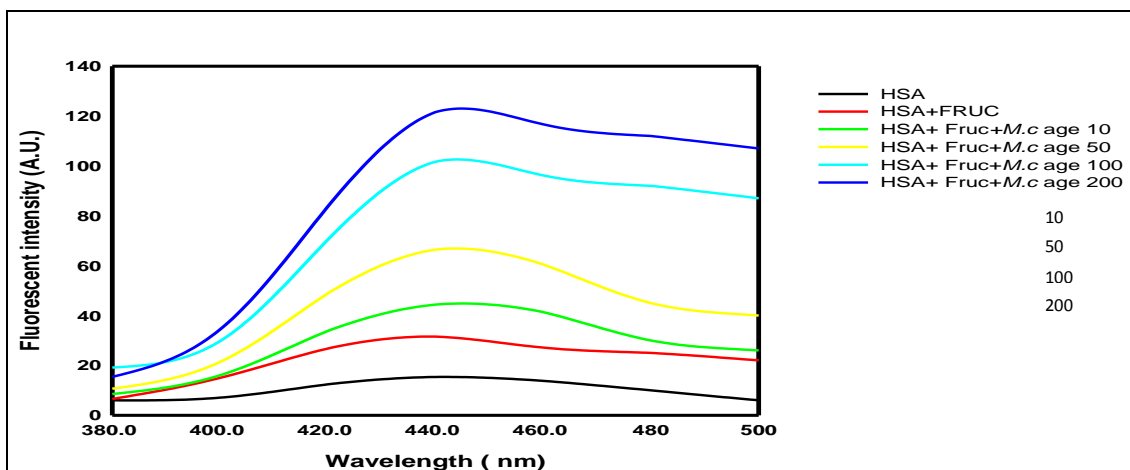


FIG. 2: AGES SPECIFIC FLUORESCENCE SPECTRA OF NATIVE, GLYCATED AND *M.CHARANTIA* L EXTRACT TREATED GLYCATED HSA SAMPLES.

Protein bound Carbonyl Groups:

Glycation of HSA with fructose led to an increase in carbonyl content. Further, treatment of glycated HSA sample with *M. Charantia* L extract was

associated with increase in protein bound carbonyl groups in a dose-dependent manner as shown in **Fig. 3**.

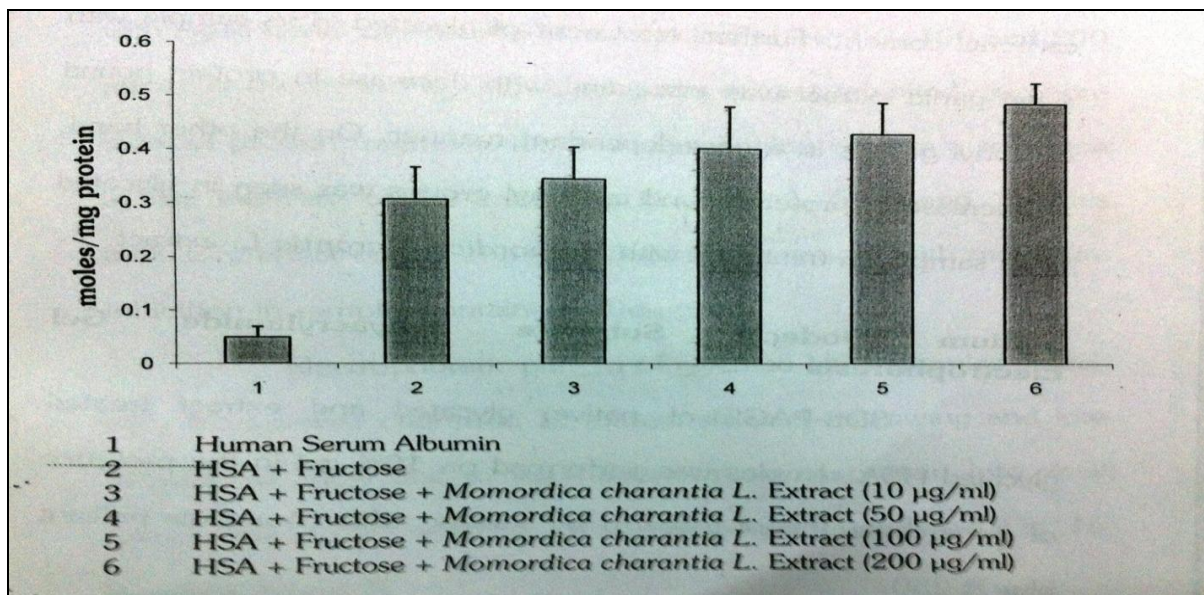


FIG. 3: DETERMINATION OF PROTEIN CARBONYLS IN NATIVE, GLYCATED AND *M.CHARANTIA* L EXTRACT TREATED GLYCATED HSA SAMPLES.

Sodium Dodecyl Sulphate Polyacrylamide Gel (SDS-PAGE) Electrophoresis:

Electrophoretic pattern of glycated HSA samples treated with *M. Charantia* L extract showed narrowing and low intensity of band at a concentration of 10, 50, 100 and 200µg/ml is shown in **Fig.4**. *M. Charantia* L extract inhibited

glycation-induced protein fragmentation and cross-linking causing a reduction in the intensity of the band (**Fig. 4**, lane 4, 5, 6 and 7). This inhibitory effect occurs in a dose-dependent manner with maximum inhibition in samples containing 200µg/ml.

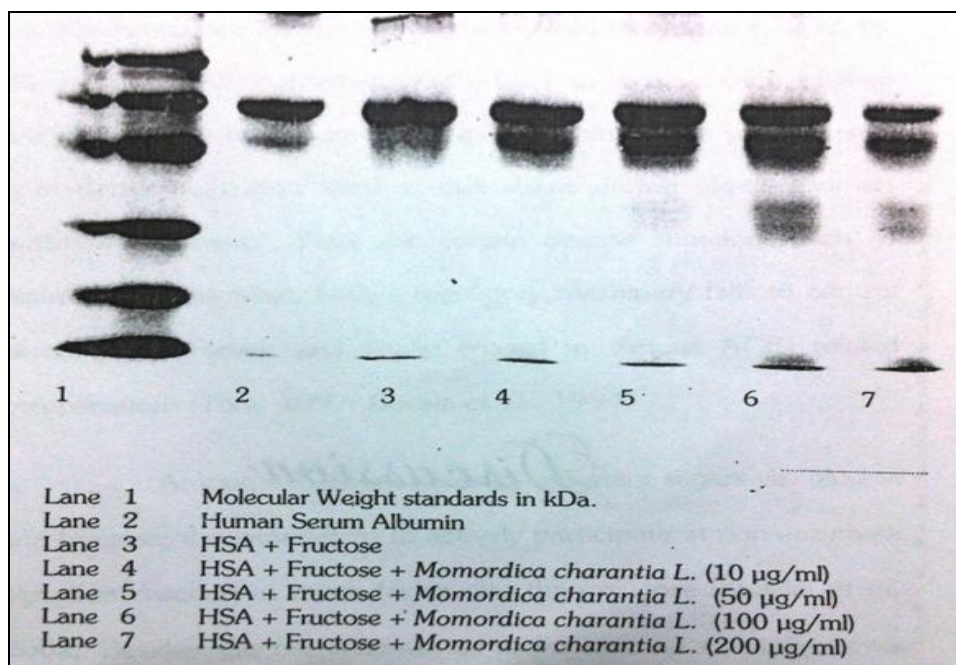


FIG. 4: SDS-PAGE OF HSA UPON *IN VITRO* GLYCATION IN THE ABSENCE AND PRESENCE OF *M.CHARANTIA* L EXTRACT

DISCUSSION: Interaction of proteins with sugars results in structural modification of the former that ultimately lead to formation of AGEs^{22, 1-2}. Higher animals have evolved various strategies to maintain the plasma sugar level thereby avoiding such complications during life-time of an individual. However, there are certain disease situations such as diabetes mellitus when body's regulatory machinery fails to control blood glucose levels and finally ensued in various AGEs related complications^{23, 3}.

A wide variety of natural anti-glycating agents have been reported²⁴⁻²⁶ & recently there has been interest in natural products with anti-glycation properties. In the present study we have studied effect of some *M. Charantia* L extract on fructose mediated glycation of model protein Human Serum Albumin.

The data of the present study suggest that co-incubation of the *M. Charantia* L. extract with HSA-fructose mixture intensify the fructose mediated glycation of HSA. The tryptophan fluorescence studies revealed that *M. Charantia* L. further dequenches fluorescence intensity of the fructose treated HSA in dose-dependent manner. It seems the *M. Charantia* L. extract causes conformational changes in the HSA structure that ultimately leads to further burying of the tryptophan residues. AGE related fluorescence further confirms above observations and suggests that *M. Charantia* L. enhances AGEs formation that ultimately results in increase in fluorescent intensity. Incidentally, *M. Charantia* L. was also found to increase reactive carbonyl compound formation that reacts with protein to form highly reactive ketoamines. The SDS-PAGE analysis of the co-incubation mixture of HSA- fructose and *M. Charantia* L. extract also supports the view that it compliments fructose mediated degradation of HSA.

On the contrary, Ghous et al reported that aqueous extract of *M. charantia* fruit pulp has pronounced DPPH (2,2-diphenylpicrylhydrazyl) and ABTS (2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid) radical scavenging potential and in the antiglycation assay it has illustrated considerable inhibitory activities against the formation of AGEs

induced by glucose with an efficacy of 75% with 150 µl of pulp extracts²⁷. Tripathi et al reported that treatment of diabetic rats with aqueous extract of *M. charantia* pulp for 30 days causing significant improvement in anti-oxidant activity (super oxide dismutase, catalase, reduced glutathione content, glutathione-s-transferase) measured in heart, liver & kidney tissues of diabetic rats²⁸.

The observed pro-glycating properties of *M. Charantia* L extract induce a sense of precaution as *M. Charantia* L has been reported to possess strong anti-diabetic effect. This study clearly suggest that, antidiabetic effect of *M. Charantia* L may be at the insulin-sugar interaction level, however some of its components can aggravate sugar mediated glycation and degradation of various proteins of the living cells.

CONCLUSION: Main aim of the study was to investigate the role of *M. Charantia* L extract in protein glycation and it has been found that *M. Charantia* L. seems to aggravate sugar mediated glycation of the protein and need further studies to pinpoint specific bioactive compounds responsible for the observed activities.

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