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ARTICLE

Effect of different artificial sweeteners on protein glycation

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ABSTRACT Glycation refers to the non-enzymatic molecular interaction between carbonyl group of sugars and amino groups of macromolecules viz. proteins, DNA, and lipids leading to the generation of Schiff's base, Amadori products, and finally converted to deleterious advanced glycation end-products (AGEs). Several diseases such as neurodegenerative or mental disorders, cardiovascular complications, as well as diabetes, and its related complications show glycated product involvement. Hyperglycemia and diabetes are the main diseases in which AGEs formation and its accumulation are enhanced and cause secondary complications. This study was performed to investigate the antiglycation and anti-aggregation potential of Food and Drug Administration-approved artificial sweeteners. The *in vitro* glycation system (BSA and glucose) was incubated along with artificial sweeteners *viz* acesulfame potassium, saccharin sodium, sucralose, aspartame, and neotame for 35 days at 37 °C. The conventional analytical methods such as browning, NBT assay, DNPH assay, and assessment of fluorescent AGEs were carried out spectroscopically to check the amount of glycation products. The presence of the mentioned artificial sweeteners in the glycation system showed inhibition of carbonyl content, total AGEs generation, and aggregation of β-amyloid structures. On day 35, acesulfame potassium reduced carbonyl content by 62.63 ± 0.91%, total AGEs generation by 49.39 ± 0.82%, and β-amyloid aggregation observed by Thioflavin-T assay by 43.45 ± 1.14%. The tested artificial sweeteners exhibited potential antiglycation and anti-aggregation activity *in vitro* in protein, BSA. They may be used as a therapeutic agent for the management of diabetes and its complications.

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Introduction

Diabetes has by now become a major metabolic and endocrinological disorder. Increased concentration of glucose over 7.0 mmol/l in blood when fasting is considered as hyperglycemia (WHO 2019). Hyperglycemia leads to the accumulation of undesired products due to an excess amount of glucose present in the body (Chiang et al. 2014). Excess level of glucose interferes by modification of protein or other biological macromolecules structure through physiological processes viz reduction, oxidation, precipitation, etc. Glycation is the multistep process in which carbonyl group of glucose interacts with an amino group of nitrogenous compounds like proteins, nucleic acids, etc. The discovery of the interaction between amines and saccharides forming the basis of the browning reaction by Louie Camille Maillard in 1912, led to a huge amount of research on glycation and its implications (Kikuchi et al. 2003). The main amino group of amino acid residues viz. lysine, hydroxylysine, arginine, and histidine interact with the carbonyl group of an acyclic reducing sugar like D-glucose, to generate a carbinolamine intermediate and an unstable imine adduct in the Maillard reaction (Schiff's base). The Schiff's base can undergo spontaneous Amadori rearrangement at physiological pH and room temperature, resulting in a more stable advanced glycation end-product (AGEs) (Schalkwijk et al. 2004). More than 100 AGEs have been identified as fluorescent and non-fluorescent AGEs, such as 3-deoxyglucosone (3DG), an intermediate to AGEs (*i.e.*, pyrraline), N^{ε}-(carboxymethyl) lysine (CML), pentosidine, methylglyoxal lysine dimer (MOLD) and glyoxal lysine dimer (GOLD) (Soboleva et al. 2017).

The carbonyl stress and oxidative stress experienced by cells in glycation via generation of free radicals ultimately promote cell or tissue damage. AGEs can be responsible for the secondary complications arising from the diabetes, neurodegenerative and cardiovascular disorders, cancer, and physiological aging. Hyperglycemia are known the key factor for protein glycation and leading to AGEs formation. These AGEs get accumulated in the cells or tissues of body which are derived from glucose-mediated glycation. The deposition of intracellular AGEs at most

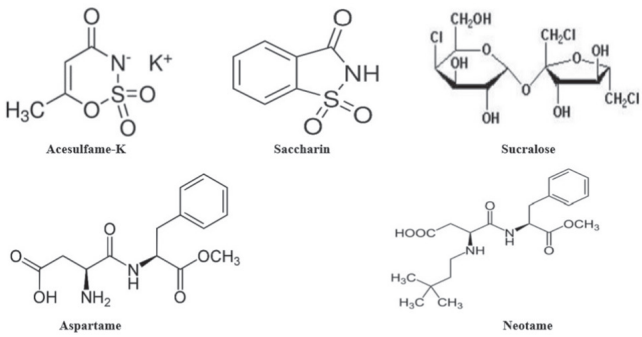

Figure 1. Chemical structure of artificial sweeteners (Dhartiben and Aparnathi 2017).

sites of diabetes complications such as retina, kidney etc. play crucial role in alteration of functioning in signaling pathways or proteins (Singh et al. 2014). AGEs also cause the intermolecular cross-linking of collagen which contribute in increasing vascular stiffness, systolic hypertension, diastolic dysfunction as well as diminished arterial and myocardial compliance (Cooper et al. 2001). Several anti-glycating agents like aminoguanidine, pyridoxamine, etc., have been reported which either inhibit the formation or reduced deleterious impact of AGEs (Deo et al. 2020; Kumar et al. 2021).

Sugar is an integral and unavoidable part of our regular diet. There are lots of low-caloric nutritional and sugar-free food products marketed for health benefits. Artificial sweeteners are popular food additives and have been utilized for preparing sweets, beverages, medicines etc. in restricted quantities. They are approximately 200-fold sweeter than natural sugars but possess lesser or no nutritional energy (Dhartiben and Aparnathi 2017). The synthetic sweetening agents are inclusively known as artificial sweeteners. Food and Drug Administration (FDA) and Food Safety and Standards Authority of India (FSSAI) have commonly approved the acesulfame K (Ace-K), aspartame (Asp), saccharin sodium (Sac), sucralose (Sucl), and neotame (Neo), advantame sweeteners based on clinical and toxicological studies within the acceptable daily intake limits (Fig. 1). Artificial sweeteners are not completely metabolized and impart minimal or no calories to the human body (Dhartiben and Aparnathi 2017; DuBois and Prakash 2012). The prevention of formation of fluorescent AGEs by sucralose in fructose-mediated glycation system was described by Deo et al. 2020. Ali et al. (2017) reported that artificial sweeteners have reversed the formation of glycation products in lysine and glucose system. Till now, however, very few reports are available on artificial sweeteners involvement in glycation process.

For management of hyperglycaemia and diabetes, food industries have used these artificial sweeteners in approved maximum amounts. People are widely consuming processed foods containing one or their combination of the approved artificial sweeteners (Deo et al. 2020). The purpose of the current study is to investigate the potential role of artificial sweeteners in protein glycation at physiological temperature for a 35 days' time period. The model glycation system, BSA and glucose, was used for quantification of Amadori products and AGEs in presence of artificial sweeteners.

Materials and Methods

Materials

Bovine serum albumin (BSA), thioflavin-T (ThT), D-dextrose (Glu) were purchased from Sigma-Aldrich. Nitroblue tetrazolium (NBT) and di-nitrophenyl hydrazine (DNPH) were procured from Hi-media (Mumbai, India). Sac, Ace-K, Sucl, Asp and Neo were obtained from Neel Chem Pvt. (Mumbai, India). All other chemicals are used of high-grade quality.

Preparation of in vitro glycation samples

In vitro glycation activity of approved artificial sweeteners viz. Sac, Ace-K, Sucl, Asp and Neo were checked with some modification by the method used by Kumar et al. (2021). The preparation of glucose-mediated glycated samples was carried out incubating glucose (100 mg/ml) with BSA (10 mg/ml) in presence and absence of artificial sweeteners viz. Sac/Ace-K/Sucl (100 mg/ml) and Asp/Neo (14.2 mg/ml) at 37 °C for 35 days. The BSA in presence of glucose was also kept as control. The reaction mixture (1 ml) was prepared containing 100 µl (BSA), 100 µl (glucose), 334 µl (Sac/Ace-K/Sucl), 500 µl (Asp/Neo) in triplicates (n = 3) from the freshly prepared stock. The prevention of bacterial contamination in prolonged incubation was checked by addition of 100 µl sodium azide (3 mM) and pH 7.0 was maintained with addition of 100 µl phosphate buffer (100 mM).

Measurement of browning

The browning measurement of protein glycation products in presence and absence of artificial sweeteners can be assessed with a spectrophotometer (Shimadzu UV 1800) in triplicates at 420 nm (Ali et al. 2022). The formula for calculation of inhibition percent was applied as below:

$$\text{Inhibition (\%)} = (\text{Ac-At})/\text{Ac} \times 100 \qquad \text{Eq. 1}$$

Where, Ac refers to the absorbance of control, and At refers to the absorbance of test sample.

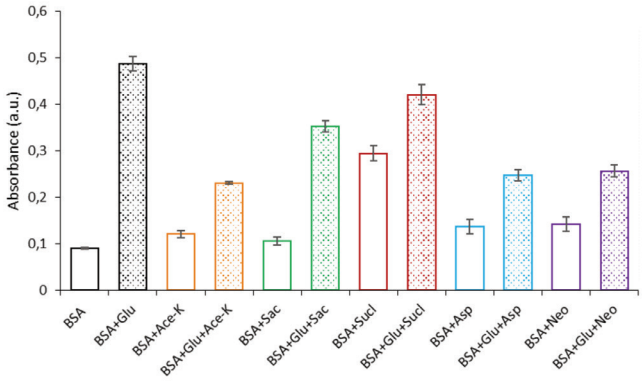

Figure 2. Measurement of browning in glycated protein sample in presence of artificial sweeteners. (n = 3 and p-value < 0.5). (BSA- bovine serum albumin, Ace-K – acesulfame potassium, Sac – saccharin sodium, Sucl – sucralose, Asp – aspartame, and Neo – neotame, a.u. – arbitrary unit).

Measurement of fructosamine content

The NBT method was employed for the evaluation of Amadori products or fructosamine content in triplicates (Ali et al. 2022). 0.1 ml NBT (0.5 mmol/l) and 10 µl glycated protein sample were incubated at 37 °C for 15 min. Measurement of optical density at 530 nm was carried out with filling of reaction mixture up to 1 ml using distilled water. The calculation of inhibition of fructosamine content was done as per Eq. 1.

Estimation of carbonyl content

The DNPH method mentioned by Meeprom et al. (2013) for evaluation of carbonyl content evaluation was employed with slight modifications in triplicates. The glycated samples (0.1 ml) in presence and absence of artificial sweeteners and 0.4 ml DNPH (10 mmol/l) were incubated in dark at 37 °C for an hour. 0.5 ml of 20% (w/v) TCA was mixed after incubation to reaction mixtures and kept in ice condition for 5 min to precipitate protein. On centrifuging for 10 min at 10000 rpm at 4 °C, the obtained pellet was allowed to wash thrice with mixture of ethanol:ethyl acetate (1:1) (500 µl). The resulting pellet was dissolved in 0.25 ml 6 M guanidine HCl. The optical density was measured at 370 nm on raising volume up to 1 ml with distilled water. The calculation of inhibition of carbonyl content was done as per Eq. 1.

Estimation of total fluorescent AGEs

Total fluorescent AGEs generated was evaluated by exciting the glycated samples at 370 nm and recording emission spectrum between 380-600 nm by Cary Eclipse Fluorescence spectrophotometer (Agilent Varian) (Kumar et al. 2021). The calculation of inhibition of total fluorescent AGEs formation was done as per Eq. 1.

Measurement of β-amyloid structure by Congo red dye and ThT method

Aggregates of β-amyloid structure are aggregation markers that can be evaluated in the glycated protein by Congo red and ThT method (Kumar et al. 2021). The glycated sample (50 µl) and 100 µM Congo red dye in 1:1 ratio was incubated in triplicates at 25 °C for 20 min. The reaction mixture's volume was brought up to 1.0 ml with distilled water and optical density was measured at 530 nm. Similarly, ThT (20 µM) was mixed to glycated samples (50 µl) treated with or without artificial sweeteners and analyzed for protein aggregates at excitation wavelength 440 nm and emission spectrum recorded between 450-600 nm using a Cary Eclipse Fluorescence spectrometer (Varian). The calculation of inhibition of aggregates of β-amyloid structure was done as per Eq. 1.

Statistical analysis

Microsoft Excel software (Microsoft Corp.) was employed for statistical analysis and Pearson correlation coefficient (R) for relationships between data of analytical assays was calculated from Pearson correlation matrix. For finding the p-value, Student's T-test was employed and results represented as Mean ± S.E of three parallel sets (n = 3). The p-value ≤ 0.05 considered as statistically significance in all analytical methods.

Results

Measurement of browning

The measurement of browning intensity showed that Ace-K inhibited the glucose-mediated glycation system by 52.66 ± 0.97%. Sac and Sucl have also decreased the browning intensity by 27.66 ± 1.22% and 13.73 ± 2.08%, respectively. Asp and Neo have shown almost similar inhibition by 49.18 ± 1.33% and 47.34 ± 1.17%, respectively (Fig. 2). Ace-K was more efficient in reducing maximum browning intensity in glycated protein than other artificial sweeteners.

Measurement of fructosamine content

The fructosamine content reduction was reduced to 19.30 ± 0.73, 24.07 ± 1.03, and 32.44 ± 1.58 µmol/mg protein in presence of Ace-K, Sac and Sucl, respectively, compared to glycated BSA (33.62 ± 0.99 µmol/mg protein). Similarly, the presence of Asp and Neo suppressed the fructosamine content to 19.69 ± 1.13 and 20.88 ± 0.95 µmol/mg protein in glycated protein, respectively (Fig. 3). Ace-K reduced the fructosamine content by 42.60 ± 0.73% then followed by Asp (41.45 ± 1.13%), Neo (37.91 ± 0.95%), Sac (28.41 ± 1.03%), and Sucl (3.51 ± 1.58%) in glycated BSA (Fig. 3).

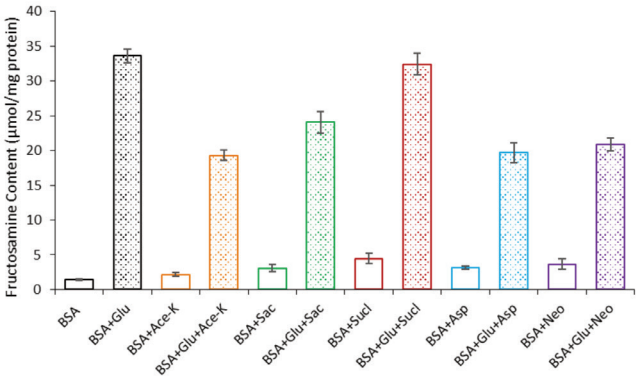

Figure 3. Measurement of fructosamine content in glycated protein sample in presence of artificial sweeteners. (n = 3 and p-value < 0.5). (BSA- bovine serum albumin, Ace-K – acesulfame potassium, Sac – saccharin sodium, Sucl – sucralose, Asp – aspartame, and Neo – neotame).

Measurement of carbonyl content

The carbonyl content was suppressed to 10.06 ± 0.91, 18.39 ± 0.85 and 25.95 ± 0.74 µmol/mg protein in presence of Ace-K, Sac and Sucl, respectively, versus glucose-mediated glycated BSA (26.90 ± 0.50 µmol/mg protein). Asp and Neo inhibited the carbonyl content to 11.61 ± 0.70 and 13.62 ± 1.01 µmol/mg protein in glycated protein. Ace-K managed to diminish carbonyl content generation by 62.63 ± 0.91%, followed by Asp (56.85 ± 0.70%), Neo (49.38 ± 1.01%), Sac (31.66 ± 0.85%), and Sucl (3.53 ± 0.74%) in glycated BSA (Fig. 4).

Estimation of total fluorescent AGEs

Generation of fluorescent AGEs was impeded by Ace-K (49.39 ± 0.82%), Sac (20.51 ± 1.27%), and Sucl (9.47 ± 1.41%) in glycated BSA, respectively. Similarly, the reduced total fluorescent AGEs formation was calculated to 45.19 ± 0.93% and 43.79 ± 1.22% in presence of Asp and Neo,

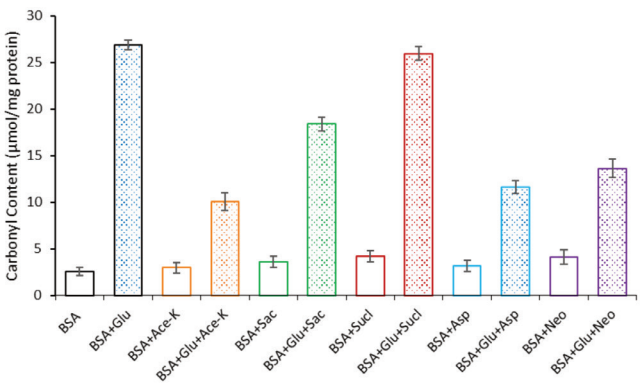

Figure 4. Measurement of carbonyl content in glycated protein sample in presence of artificial sweeteners. (n = 3 and p-value < 0.5). (BSA- bovine serum albumin, Ace-K – acesulfame potassium, Sac – saccharin sodium, Sucl – sucralose, Asp – aspartame, and Neo – neotame).

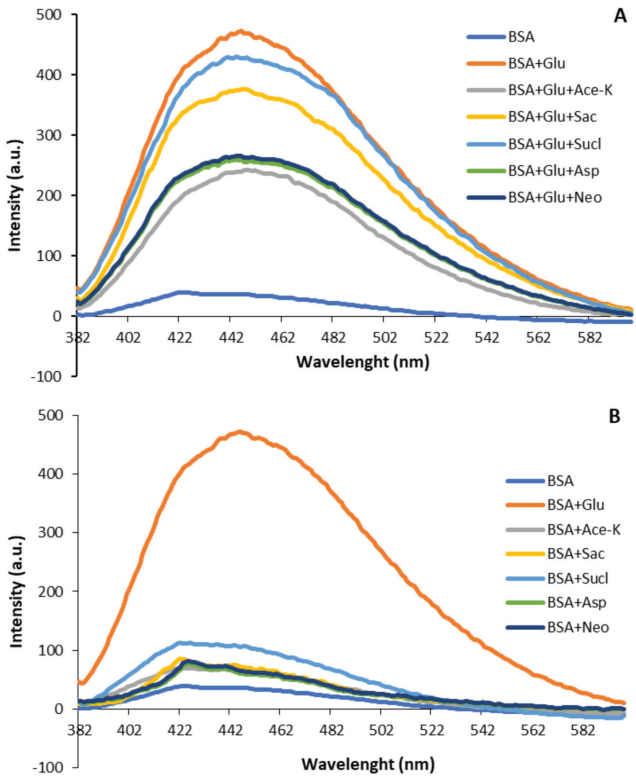

Figure 5. Spectrum of total AGEs formation; (A) glycated protein in presence of artificial sweeteners, (B) native protein in presence of artificial sweeteners. (BSA- bovine serum albumin, Ace-K – acesulfame potassium, Sac – saccharin sodium, Sucl – sucralose, Asp – aspartame, and Neo – neotame; a.u. – arbitrary unit).

respectively than the glucose mediated glycation system (Fig. 5A). The spectrum of total fluorescent AGEs formation of BSA in presence of all studied artificial sweeteners showed the similar intensity to the intensity of BSA (Fig. 5B). Thus, the formation of total fluorescent AGEs in glycated protein was suppressed by Ace-K.

Measurement of β-amyloid structure by Congo red and thioflavin-T (ThT) dye

The accumulation of β-amyloid structure can observe involvement of AGEs generated in glucose mediated glycation system. The aggregation of glycated BSA was impeded in the presence of Ace-K (42.75 ± 1.73%), Sac (34.25 ± 2.03%), Asp (39.47 ± 1.26%), and Neo (38.65 ± 0.96%) versus glycated BSA as control (Fig. 6). Similarly, the spectrum of aggregated β-amyloid structure in the ThT dye assay showed the decrease of glycated protein aggregation by 43.45 ± 1.14%, 37.36 ± 1.84%, 41.83 ± 1.20%, and 40.68 ± 1.06% in presence of Ace-K, Sac, Asp, and Neo, respectively (Fig. 7A). These artificial sweeteners except Sucl along with native BSA have shown almost similar intensity of aggregation as BSA alone (Fig. 7B).

Table 1. Pearson's correlation coefficient between browning, fructosamine content, carbonyl content, total AGEs and β-amyloid aggregation.

	Browning	Fructosamine content	Carbonyl content	Total AGEs	β-amyloid aggregation
Browning	1.000				
Fructosamine Content	0.921	1.000			
Carbonyl content	0.919	0.991	1.000		
Total AGEs	0.875	0.989	0.980	1.000	
β-amyloid aggregation	0.929	0.983	0.973	0.982	1.000

However, Sucl was failed to reduce the aggregation of protein. Glycation and aggregation were substantially prevented in the presence of Ace-K.

Correlation analysis between various antiglycation potential of artificial sweeteners

The relationship between the independent analytical methods applied to test the antiglycation and anti-aggregation potential of artificial sweeteners was examined by the Pearson correlation analysis. The total AGEs formation was positively correlated with carbonyl and fructosamine content. The β-amyloid aggregation and total AGEs formation were strongly correlated by carbonyl and fructosamine content generation (Table 1). Thus, the analytical data of both protein glycation and protein aggregation showed that artificial sweeteners suppressed the glucose-mediated glycation and aggregation process.

Discussion

Neurodegenerative disorders (Alzheimer's and Parkinson's disease), diabetes and its vascular complications and early onset of aging have been correlated with the hyperglycemic condition and oxidative stress through free radicals' generation and tissue damage (Ali et al. 2017; Hammoda 2013; Kim and Kim 2003). The changes in the structure, biological activity, and half-life of proteins cause mutations in nucleic acids, as well as affect transport and signaling processes by causing damage to lipids in the membrane (Tupe et al. 2015). Many synthetically manufactured AGE inhibitors have showed promising outcomes *in vitro* and in diabetic animals, but are still in the clinical trial phase due to negative effects (Tupe et al. 2015; Kumar et al. 2021).

In the patients of elevated blood glucose level, gen-

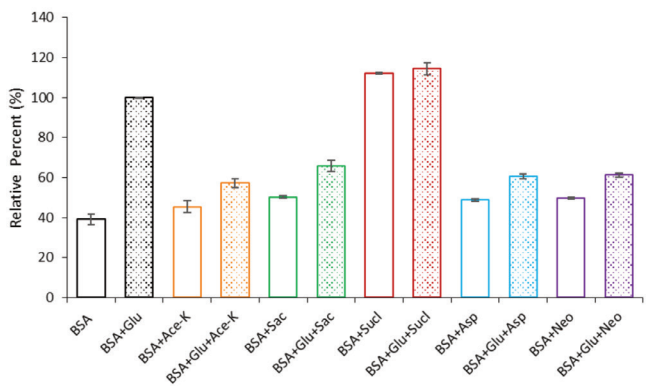

Figure 6. Measurement of β-amyloid structure by Congo red dye in glycated protein in presence of artificial sweeteners. (n = 3 and p-value < 0.5). (BSA- bovine serum albumin, Ace-K – acesulfame potassium, Sac – saccharin sodium, Sucl – sucralose, Asp – aspartame, and Neo – neotame).

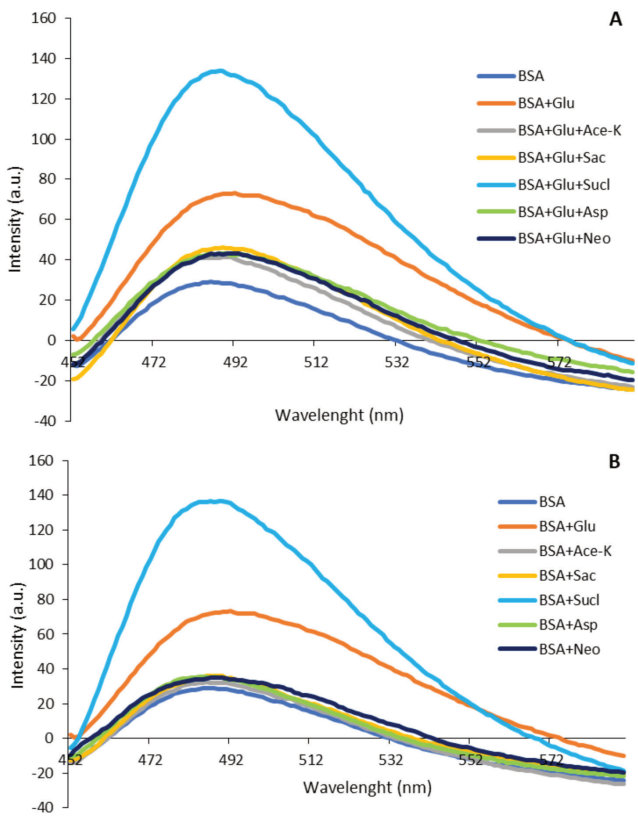

Figure 7. Spectrum of β-amyloid structure by thioflavin-T (ThT) dye; (A) glycated protein in presence of artificial sweeteners, (B) native protein in presence of artificial sweeteners. (BSA- bovine serum albumin, Ace-K – acesulfame potassium, Sac – saccharin sodium, Sucl – sucralose, Asp – aspartame, and Neo – neotame; a.u. – arbitrary unit).

eration of free radicals, leading to damage at cell or tissue level, was noticed many times (Glodin et al. 2006). The artificial sweeteners have been used in cold drinks, chocolates, sweets, etc. by replacing the natural sugars for the management diabetes or hyperglycemic conditions in patients (Dhartiben and Aparnathi 2017). Artificial sweeteners viz. Ace-K, Sac, Sucl, Asp, Neo, cyclamate and their different combinations are frequently used by patients affected by hyperglycemia and/or diabetes for low-caloric intake. The high dose consumption of these sweeteners has some side-effects like cardiovascular disorders, mental disorders, brain tumors, and bladder cancer, etc. (Kant 2005; Weihrauch and Diehl 2004).

BSA has physiological relevance to human serum albumin which is the reason why it was considered for the elucidation of effects of artificial sweeteners on protein glycation. The artificial sweeteners exerted inhibitory effect on the generation of early, intermediate, and late-stage products of glucose-glycation process. The generation of brownish color, fructosamine or Amadori products, and carbonyl content was reduced by Ace-K. The Sac, Asp, and Neo also showed considerable suppression of early glycated products generation. Ali et al. (2022) reported that *Stevia rebaudiana*, a sweetener, showed the suppression of early and late-stage products in fructose-mediated glycation. However, Sucl failed to reduce the glycated product formation. The mono-carbonyl sugar, glucose, is less reactive than the fructose, ribose, and methylglyoxal, a di-carbonyl compound (Schalkwijk et al. 2004). In diabetes patients' generation of Schiff's base, fructosamine or Amadori products, as well as different kinds of carbonyl or di-carbonyl compounds, can be initiated through hyperglycemic conditions and oxidative stress. This condition should be managed by means of drugs or compounds having antiglycation and antioxidant properties (Tupe et al. 2015). The generated glycated products in early stage or intermediate-stage are converted into AGEs which interfere with the functioning of native proteins and other biomolecules. The non-functional or disrupted native proteins get accumulated (Tupe et al. 2015). The spectrum of total AGEs formation showed that Ace-K reduced total AGEs generation most strongly, followed by Neo, Asp, and Sac.

Further, the intensity of glycated protein aggregation reduced in presence of Ace-K, Asp, Neo, and Sac in decreasing order. In our study we have used Congo red and ThT methods for the analysis of protein aggregation because of their differential specificity. The dye-binding tests frequently are used to investigate the putative inhibitors of amyloid fibril formation and their aggregation. These dye-based assays may provide false positive results due to similar binding sites as of inhibitors. For avoiding this, either dye-free assays/techniques (CD, DLS, microscopy, etc.) should be parallelly performed or use of more than one dye-based assay with different spectral and structural properties, for confirming the protein aggregation results (Jameson et al. 2005; Schmuck et al. 2005). Ace-K and Sac have been reported not to be metabolized in the body and be inert in nature (Dhartiben and Aparnathi 2017). However, Asp and Neo are broken down and metabolized in the body and provide some calories (Dhartiben and Aparnathi 2017; DuBois and Prakash 2012). The results of the present comparative study of sweeteners in glycation process are supported by earlier reports on the glycation process in presence of sweeteners (Ali et al. 2017). The biochemical mechanism of artificial sweeteners in glycation process still does not have clear evidence in literature. However, preliminary studies have been published that suggest that some of the artificial sweeteners cause inhibition of both early and late-stage glycated products in serum protein glycation (Ali et al. 2017, Ali et al. 2022).

Conclusion

Certain neurodegenerative and cardiovascular disorders, especially when complications of diabetes mellitus, are directly or indirectly correlated with the generation of glycated products and AGEs. In the current study, Ace-K showed the strongest antiglycation potential of all investigated artificial sweeteners, but Sac, Asp, and Neo also showed noticeable antiglycation activity in the *in vitro* glucose-mediated glycation system. From these research findings, Ace-K can be a potential candidate for management of diabetes and its complications in patients. Further, Ace-K sweetener need to be investigated pharmacologically for its use as therapeutic against diabetes and glycation induced pathologies.

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References

Ali A, More TA, Hoonjan AK, Sivakami S (2017) Antiglycating potential of acesulfame potassium: an artificial sweetener. Appl Physiol Nutr Metab 42(10):1054-1063.

Ali A, Shahu R, Balyan P, Kumari S, Ghodmare R, Jobby R, Jha P (2022) Antioxidation and antiglycation properties

of a natural sweetener: *Stevia rebaudiana*. Sugar Tech 24(2):563-575.

Chiang JL, Kirkman MS, Laffel LMB, Peters AL (2014) Type 1 diabetes through the life span: a position statement of the American Diabetes Association. Diabetes Care 37(7):2034-2054.

Cooper ME, Bonnet F, Oldfield M, Jandeleit-Dahm K (2001) Mechanisms of diabetic vasculopathy: an overview. Am J Hypertens 14:475-486.

Deo P, Chern C, Peake B, Tan SY (2020) Non-nutritive sweeteners are in concomitant with the formation of endogenous and exogenous advanced glycation end-products. Int J Food Sci Nutr 71(6):706-714.

Dhartiben BK, Aparnathi KD (2017) Chemistry and use of artificial intense sweeteners. Int J Curr Microbiol App Sci 6:1283-1296.

DuBois GE, Prakash I (2012) Noncaloric sweeteners, sweetness modulators and sweetener enhancers. Annu Rev Food Sci Technol 3:353-380.

Goldin A, Beckman JA, Schmidt AM, Creager MA (2006) Advanced glycation end products: sparking the development of diabetic vascular injury. Circulation 114(6):597-605.

Hammoda HM, Ghazy NM, Harraz FM, Radwan MM, ElSohly MA, Abdallah II (2013) Chemical constituents from *Tribulus terrestris* and screening of their antioxidant activity. Phytochemistry 92:153-159.

Jameson LP, Smith NW, Dzyuba SV (2012) Dye-binding assays for evaluation of the effects of small molecule inhibitors on amyloid (aβ) self-assembly. ACS Chem Neurosci 3(11):807-819.

Kant R (2005) Sweet proteins – Potential replacement for artificial low-calorie sweeteners. Nutr J 4:5.

Kikuchi S, Shinpo K, Takeuchi M, Yamagishi S, Makita Z, Sasaki N, Tashiro K (2003) Glycation - a sweet tempter for neuronal death. Brain Res Rev 41(2-3):306–323.

Kim HY, Kim K (2003) Protein glycation inhibitory and antioxidative activities of some plant extracts *in vitro*. J Agric Food Chem 51(6):1586-1591.

Kumar D, Desa A, Chougle S, Bhatkalkar SG, Sachar S, Selvaa Kumar C, Ali A (2021) Evaluation of the antiglycating potential of thymoquinone and its interaction with BSA. J Biomol Struct Dyn 40(18):8455-8463.

Meeprom A, Sompong W, Chan CB, Adisakwattana S (2013) Isoferulic acid, a new anti-glycation agent, inhibits fructose- and glucose-mediated protein glycation *in vitro*. Molecules 18(6):6439-6454.

Schalkwijk CG, Stehouwer CDA, van Hinsbergh VWM (2004) Fructose-mediated non-enzymatic glycation: sweet coupling or bad modification. Diabetes Metab Res Rev 20:369-382.

Schmuck C, Frey P, Heil M (2005) Inhibition of fibril formation of Aβ by guanidiniocarbonyl pyrrole receptors. Chem Bio Chem 6:628-631.

Singh VP, Bali A, Singh N, Jaggi AS (2014) Advanced glycation end products and diabetic complications. Korean J Physiol Pharmacol 18(1):1-14.

Soboleva A, Vikhnina M, Grishina T, Frolov A (2017) Probing protein glycation by chromatography and mass spectrometry: analysis of glycation adducts. Int J Mol Sci 18(12):2557.

Tupe RS, Sankhe NM, Shaikh SA, Phatak DV, Parikh JU, Khaire AA, Kemse NG (2015) Aqueous extract of some indigenous medicinal plants inhibits glycation at multiple stages and protects erythrocytes from oxidative damage-an *in vitro* study. J Food Sci Technol 52(4):1911-1923.

Weihrauch MR, Diehl V (2004) Artificial sweeteners - do they bear a carcinogenic risk? Ann Oncol 15(10):1460-1465.

World Health Organization (2019) Classification of diabetes mellitus. Retrieved from https://www.who.int/publications/i/item/classification-of-diabetes-mellitus