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Ph.D. Thesis Summary

Studies on the Artificial Sweeteners in the Glycation of Protein

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Diabetes has become a major metabolic and endocrine disorder. Recent estimates indicated that 536.6 million adults were living in the world with diabetes in the year 2021. From 2000 to 2021, there will be a rise in the number of diabetes patients in the adult population globally, from 4.6 to 9.8%. The scenario in India also follows trends similar to those of developed and developing countries. India has been recognized as the "diabetes capital or epicentre". India had 65.1 million estimated diabetes cases in 2013, which is expected to increase to 109 million in 2035.

All types of diabetes have common features, like hyperglycemia and glucotoxicity. Glycation is a process in which reducing sugar binds to biomolecules' sensitive free amino groups and forms Schiff's base, Maillard products and advanced glycation end products (AGEs). The AGEs form a stable complex and heterogeneous group of molecules, affecting many intracellular functions through signalling pathways and extracellular protein cross-linkage. Glycation is implicated in ageing and neurodegenerative diseases due to its ability to induce protein cross-linking, aggregation and precipitation, misfolding, fibril and amyloid formation. However, which product(s) is/are involved in these complications and the mechanism is still unclear.

Patients suffering from diabetes are advised to take a sugar-free diet for diabetes management; for this and other health benefits, sweeteners are preferred in the food and pharmaceutical industries. Some of the very commonly used sweeteners (natural and artificial) are aspartame, saccharin, neotame, stevia, sucralose, etc., approved by the U.S. Food and Drug Administration.

This research investigates the impact of the U.S. FDA and FSSAI-approved artificial sweeteners artificial sweeteners, viz. Acesulfame-Potassium (Ace-K), aspartame, neotame, saccharine and sucralose, on glycation processes and protein aggregation, with a focus on two model glycation systems: Glucose-BSA and methyl glyoxal-BSA. The study also explores the role of antioxidants and vitamins in the glycation process and protein aggregation. Glycation, a process where sugars react with proteins, can form harmful products and impact protein structures.

The study begins by preparing glycated samples using BSA exposed to various compounds, including artificial sweeteners, at different temperatures and durations. Out of the studied artificial sweeteners, Ace-K significantly reduces the formation of glycation products, such as browning, Amadori products, carbonyl content and advanced glycation end-products (AGEs), in both glucose and methyl glyoxal glycation systems. The inhibitory effect of Ace-K surpasses other artificial sweeteners and is comparable to aminoguanidine (AG), a known glycation inhibitor.

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Moreover, the research evaluates the impact of Ace-K on the glycation-induced aggregation of β-amyloid structures, which are implicated in various diseases, including Alzheimer's. The Ace-K demonstrates a remarkable reduction in protein aggregation compared to the glycated system, as indicated by protein aggregation index (PAI) measurements and Congo red/Thioflavin-T dye assays. The inhibitory effect of Ace-K extends to thermal-treated glycation systems and methyl glyoxal-mediated glycation.

The study also delves into the structural alterations caused by glycation and demonstrates that Ace-K helps preserve the structural integrity of proteins. Free lysine and arginine modifications, loss of esterase-like activity and alterations in CD spectra characteristic of glycation are significantly mitigated in the presence of Ace-K.

Additionally, the research explores the influence of antioxidants and vitamins on glycation and protein aggregation. Thymoquinone emerges as a potent inhibitor, significantly reducing glycation product formation and β-amyloid structure aggregation. Other antioxidants, such as ferulic acid and pyridoxal-5-phosphate, exhibit inhibitory properties, while ascorbic acid shows the least attenuation.

In conclusion, acesulfame-potassium, particularly Ace-K, proves to be a robust inhibitor of glycation and protein aggregation in both glucose and methyl glyoxal glycation systems. Its potential therapeutic applications for managing diabetes and related complications are underscored. Thymoquinone also exhibits notable inhibitory properties, suggesting avenues for further exploration in *in vivo* studies. The research sheds light on artificial sweeteners and natural compounds as potential interventions in glycation-associated disorders, opening avenues for future research and development in diabetes management.

KEYWORDS

Advanced glycation end-product (AGEs), aggregation, artificial sweeteners, acesulfame-potassium (Ace-K), β-amyloid structures, bovine serum albumin, carbonyl stress, diabetes, glycation

KEY CONTRIBUTIONS

- Artificial sweeteners showed their role in preventing the formation of glycation products and their downstream process, like aggregation of β-amyloid structures. The extent of the generation of glycation products at every stage of glycation was assessed
- The present study provided many interesting and strong lines of evidence about the role of artificial sweeteners, specifically acesulfame-potassium, in glycation, protein aggregation and their structural integrity

FUTURE DIRECTIONS

Artificial sweeteners still have scope for enhancement to conduct *in vitro* and *in vivo* experimentation studies for human welfare. The execution of such experimental studies is restricted due to ethical concerns and a lack of sophisticated technical resources. The interaction mechanism of AGEs with RAGEs at the cellular level can be conducted for their role in metabolic pathways. For more comprehension and correlation of generated AGEs, conducting *in vivo* model studies to explore their potential targets and receptor sites would be appropriate. The experiments can be conducted to develop the AGE as a marker for therapeutic use to detect specific complications in the body.

The WHO has recently published guidelines on the controlled use of artificial sweeteners for obesity and the management of low-caloric diets due to their probable association with cancer. Therefore, conducting experimental studies to check their role in association with complications and disorders in long-term use becomes crucial. It is also essential to evaluate their role in toxicological studies in a concentration and time-dependent manner. Therefore, we can further assess the role of artificial sweeteners in various other diseases and disorders for health safety.

CONTRIBUTION OF EACH AUTHOR

Name of student: Dinesh Kumar, Research Student, Department of Life Sciences, University of Mumbai, Vidyanagari, Santacruz (East), Mumbai, Maharashtra-400098, India.

Contribution: Conceptualization, data collection, analysis and interpretation and draft writing.

Name of Supervisor: Dr. Ahmad Ali, Assistant Professor, Department of Life Sciences, University of Mumbai, Vidyanagari, Santacruz (East), Mumbai, Maharashtra-400098, India.

Contribution: Conceptualization, analysis and interpretation, supervision and review.

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DECLARATION LETTER

Subject: Declaration of Intent to Publish Thesis Summary in Science Digest

Dear Editor,

I, Mr. Dinesh Kumar, hereby declare on behalf of all the authors involved in the research, that we have reached a unanimous agreement to publish the summary of our thesis, titled "Studies on the Artificial Sweeteners in the Glycation of Protein," in Science Digest.

This research was conducted at the Biomolecular Laboratory, Department of Life Sciences, University of Mumbai, India, under the supervision of Dr. Ahmad Ali, during the academic year 2023. The study represents the culmination of Dinesh Kumar's Ph.D. research project and we are excited to share the key findings with the global scientific community through the esteemed platform of Science Digest.

This declaration confirms that all co-authors have been made aware of and have consented to the publication of the thesis summary in Science Digest. Furthermore, we affirm the accuracy and completeness of the information provided in the submission.

Thank you for considering our work for publication.

Sincerely,

Author 1:	Dinesh Kumar	Signature:	
Author 2:	Ahmad Ali	Signature: