ISSN: 2633-4828 Vol. 6 No.1, January, 2024

International Journal of Applied Engineering & Technology

SORBITOL ACCUMULATION AND ADVANCED GLYCATION END PRODUCT MODULATORS ACTIVITIES OF HESPERIDIN

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ABSTRACT

Introduction: *Hespiridin is a flavanoid compound found abundantly in citrus fruits and is investigated for its modulatory effect on sorbitol accumulation and advanced glycation end products which are 2 important factors associated with diabetes and its complications.*

Aim: *To evaluate the modulatory effect of Hesperidin on sorbitol accumulation and advanced glycation end product modulatory activities.*

Materials and methods: *Advanced glycation end product (AGE)assay, Aldose reductase inhibitory activity, sorbitol accumulation inhibition assay.The properties of Hespiridin was compared with the standard Metformin and Aminoguanidine.*

Results: *The results showed significant inhibition of sorbitol accumulation and inhibition of reductase activity and AGEs.Several mechanisms appear to be involved in hyperglycemia-mediated oxidative stress, such as glucose autoxidation, protein glycation, and the formation of AGEs.*

Conclusion: *Preliminary studies suggest that hesperidin may have modulatory effects on sorbitol accumulation and AGE formation, further research is necessary to elucidate the underlying mechanisms and evaluate the clinical relevance of these findings.*

Keywords: AGEs, aldose reductase, complications, diabetes, flavanoids, hespiridin, hyperglycemia, sorbitol.

INTRODUCTION

Hyperglycemia, a symptom of the chronic metabolic illness diabetes mellitus that can affect many different organs and systems in the body, can cause a number of issues. The buildup of sorbitol and the production of advanced glycation end products (AGEs) are two crucial features connected to diabetes problems.Through the polyol pathway, extra blood glucose in diabetes can be converted to sorbitol. Aldose reductase, an enzyme, converts glucose to sorbitol in this route. The sugar alcohol sorbitol builds up in several tissues, including the kidneys, the eyes, and the nerves. Sorbitol buildup can result in osmotic stress, cellular damage, and oxidative stress, which can all contribute to the emergence of diabetic problems.When sugars interact with proteins, lipids, or nucleic acids in a process known as glycation, complex compounds known as AGEs are created. Without enzymatic regulation, this process takes place and creates stable, cross-linked compounds. AGEs can build up in tissues and interfere with the structure and operation of several molecules, including proteins. These altered proteins and molecules have the potential to cause tissue damage, oxidative stress, and inflammatory reactions, which can advance diabetic complications.(1)

The citrus fruits, oranges and lemons are the main sources of hesperidin, a naturally occurring flavonoid. Due to their well-known anti-oxidant and anti-inflammatory capabilities, flavonoids are good candidates to fight the oxidative stress and inflammation linked to diabetes and its consequences.Hesperidin may serve as an inhibitor of aldose reductase, the enzyme that converts glucose to sorbitol in the polyol pathway, according to studies. Hesperidin may lessen the buildup of sorbitol in tissues and lessen the osmotic stress and cellular damage brought

on by its excess by inhibiting aldose reductase.Antioxidant qualities of hesperidin may also influence AGE development. Hesperidin can reduce oxidative stress, a major factor in the production of AGE, by scavenging free radicals and reactive oxygen species (ROS). Hesperidin may also disrupt the glycation process by inhibiting the synthesis of some AGEs or degrading already-existing AGEs, which would lessen their damaging effects on tissues.(2)

Significant factors in the emergence and progression of diabetes problems include sorbitol buildup and the production of advanced glycation end products. Hesperidin has potential as a medicinal agent to address these elements of diabetes problems because it is a natural flavonoid with antioxidant and possible inhibitory activities against aldose reductase. To completely comprehend the mechanisms and possible advantages of hesperidin in avoiding or alleviating diabetes problems linked to sorbitol accumulation and AGE formation, more research is necessary. Further preclinical and clinical research is required to ascertain the efficacy and safety of hesperidin as a treatment agent for problems associated with diabetes.(3)

The aim of this study is to evaluate the modulatory effect of Hesperidin on sorbitol accumulation and advanced glycation end product modulatory activities. The objectives of this study include to investigate the effect of Hespiridin on formation of advanced glycation end products and to evaluate the inhibitory effect of Hespiridin on aldose reductase enzyme activity and to also assess the effect of Hespiridin on sorbitol accumulation.(4)

MATERIALS AND METHODS:

The Duration of the Study was About 2 Months.

Chemicals and Reagents

Hesperidin, Aminoguanidine hydrochloride, Metformin and was procured from TCI Chemicals, India. DL-glyceraldehyde, D-glucose, Fructose, lithium sulphate, NADPH, NADP, dimethyl sulphoxide (DMSO), sorbitol, bovine serum albumin, perchloric acid, ammonium sulphate, Tris-HCl, EDTA, sucrose and sorbitol dehydrogenase were purchased from Sigma aldrich (St Louis, MO, USA). All other chemicals of analytical grade were obtained from Himedia, India and SRL chemicals, India.

Advanced Glycation end Product (AGE) Assay (Harris et al., 2011)

Advanced glycation end products (AGEs) are formed by non-enzymatic glycosylation of proteins that enhance vascular permeability in both micro and macro vascular structures by binding to specific macrophage receptors. The compound Hesperidin were evaluated for its activity on AGEs formation at different concentrations of 2.5-25µM. AGE reaction mixture was constituted as follows; 1 mg/mL bovine serum albumin in 50mM sodium phosphate buffer (pH 7.4) and 0.02% sodium benzoate into 0.2M fructose and 0.2M glucose. The reaction mixture (2.75mL) was treated with different concentrations of Hesperidin (2.5-25µM). Amino guanidine was used as positive control. After incubating at 37°C for 3 days, the fluorescence intensity of the reaction was determined at excitation and emission wavelengths of 350 nm and 450 nm, respectively, using Biotek synergy multi-mode reader, USA. The percentage activity was calculated with respect to solvent control.

Determination of Aldose Reductase Inhibition (Reddy et al., 2011)

A total of 531µL of 0.1 M potassium buffer (pH 7.0), 90µL of NADPH solution (1.6 mM in potassium buffer), 90µL of recombinant human aldolase reductase (AR) (6.5U/mg) (Sigma, USA - SRP6371-100UG), 90µL of ammonium sulphate solution (4 M in potassium buffer), and 90 µL of DL-glyceraldehyde (25 mM in potassium buffer) were mixed with 9µL of different concentrations of Hesperidin (2.5-25µM) in a cuvette, and the activity of AR was assessed spectrophotometrically by measuring the decrease in NADPH absorbance at 340 nm for 3 min using a spectrophotometer (Biotek Synergy H4 multimode reader, USA). Metformin was used as positive control. The inhibition of AR (%) was calculated using the following equation: $(1 - (\triangle A \text{ sample/min}) - (\triangle A \text{ blank/min})/(\triangle A \text{ control/min}) - (\triangle A \text{ blank/min})) \times 100\%$, where $\triangle A$ sample/min is the decrease in absorbance over 3 min with reaction solution, test sample, and substrate, and $\triangle A$ control/min without the test sample.

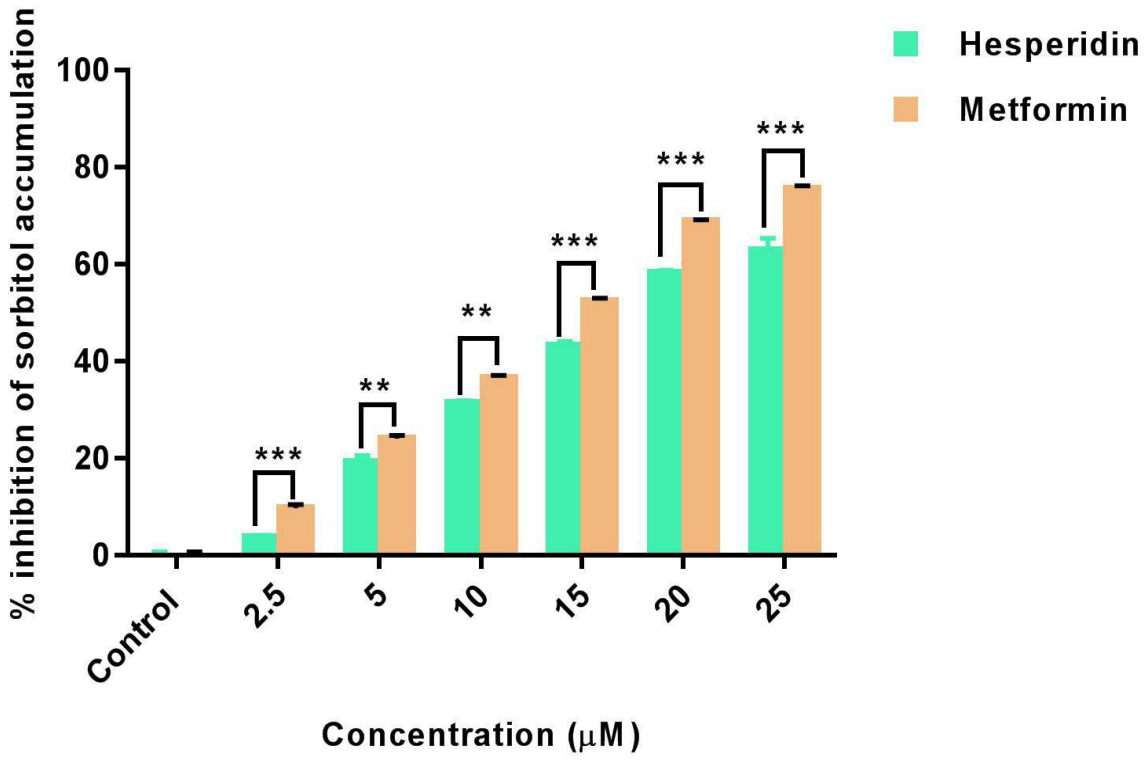
Sorbitol Accumulation Inhibition Assay (Malone et al., 1980)

5ml of blood was collected into heparinized tubes from healthy volunteers after an overnight fast. The blood was immediately centrifuged at 2000rpm for 5min, 4ºC to separate the erythrocytes from the plasma. After discarding the plasma and buffy coat, add isotonic saline (0.9% NaCl) equal to twice the volume of the erythrocytes and centrifuged at 2000rpm for 10min. Washed RBCs were suspended in Hank's balanced salt solution [HBSS] (pH 7.4) to a ratio of 1:10. Samples were incubated at 37°C for 3 h under normal (5.5mM) and high glucose (55mM) conditions. The effect of Hesperidin on sorbitol accumulation was evaluated by incubating the RBC with different concentrations of the Hesperidin (2.5-25µM). At the end of incubation periods, RBC were centrifuged, washed with saline and again centrifuged. Red cell was precipitated with cold 6% perchloric acid to the ratio of 1:3. The homogenate was centrifuged at 2000rpm at 4°C for 10 min and the pH of the supernatant was adjusted to 3.5 with 0.5M potassium carbonate. The sorbitol content of the supernatant was measured by fluorometric method. In brief, the reaction mixture contained the appropriate protein-free supernatant, 50mM glycine buffer (pH 9.4), 0.2mM NAD+, and 1.28U/ml sorbitol dehydrogenase. The mixture was incubated at 37°C for 30 min, and the relative fluorescence due to NADH was measured by a fluorescence spectrometer at an excitation wavelength of 366 nm and an emission wavelength of 452 nm.

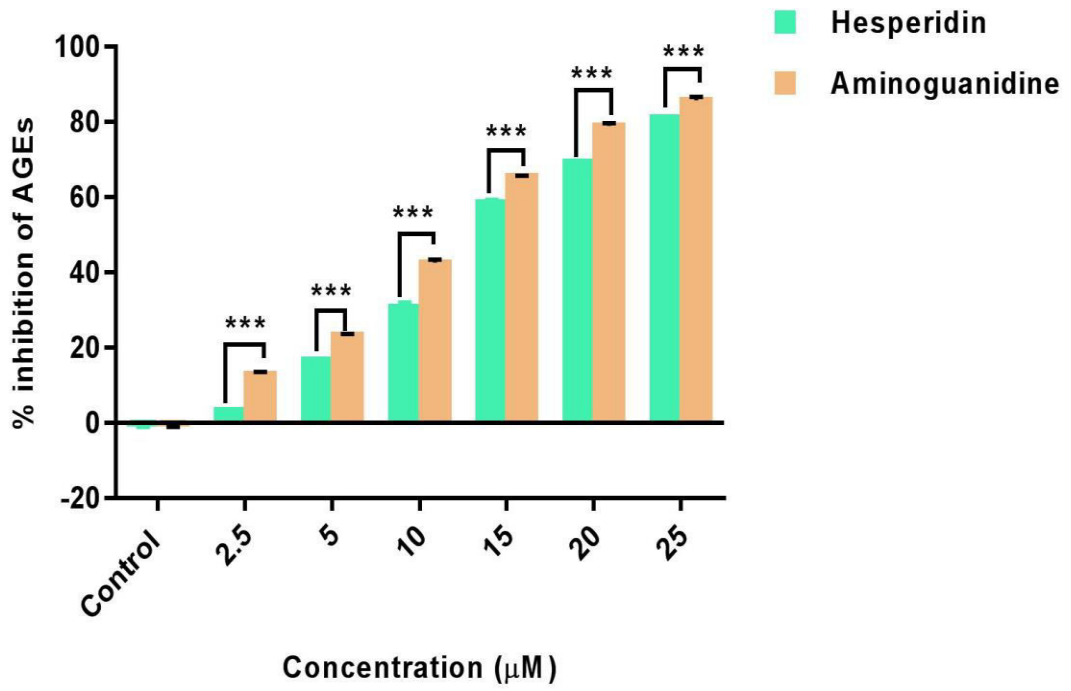
Statistical Analysis

Data were analysed using Graphpad prism (version 7.0). The results were expressed as Mean±SEM and the IC_{50} values were obtained from the linear regression plots. Two-way ANOVA was used to assess differences between means at $p<0.001$ level of significance. The means were compared with standards groups using Holm-Sidak Test.

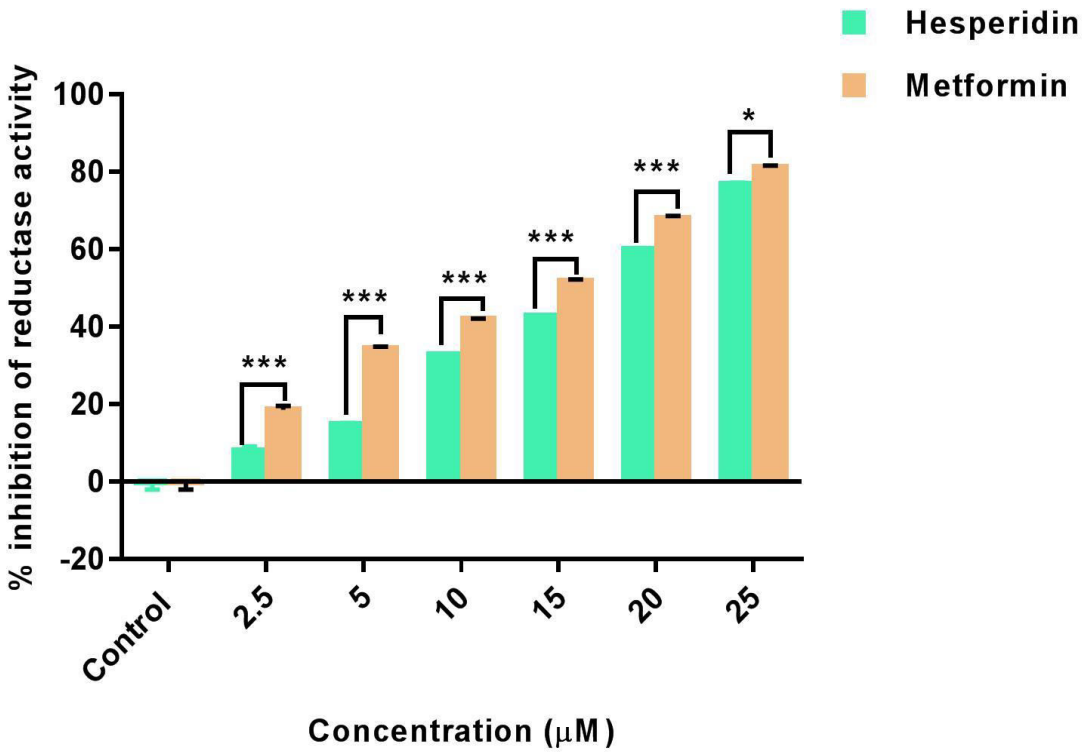
RESULTS:



Graph 1: Sorbitol accumulation



Graph 2: Advanced glycation end products activity.



Graph 3: Aldolase reductase activity

DISCUSSION

The results showed significant inhibition of sorbitol accumulation, inhibition of reductase activity and inhibition of AGEs.The above properties of Hesperidin were compared with the standard.i.e Metformin and Aminoguanidine.Three different graphs were plotted.In the first graph, percentage inhibition of sorbitol accumulation was plotted in the Y axis against concentration on the X axis.In the second graph percentage inhibition of AGEs was plotted in the Y axis against concentration on the X axis.In the third graph,percentage inhibition of reductase activity was plotted in the Y axis against concentration on the X axis.(5)

It has been suggested that free radicals and oxidation reaction are directly involved in glucose-mediated modification of proteins.Several mechanisms appear to be involved in hyperglycemia-mediated oxidative stress, such as glucose autoxidation, protein glycation, and the formation of AGEs.(6) Oxidative stress plays a critical role in the formation of AGEs, as reactive oxygen species (ROS) can promote glycation reactions. By acting as an antioxidant, hesperidin may help neutralise ROS and mitigate the oxidative stress that leads to AGE formation.This, in turn, could have implications for preventing or reducing the development of AGE-related complications in various diseases.(7,8)

According to a previous research by Asjad et al, hesperidin in combination with insulin not only attenuated the diabetic condition but also reversed neuropathic pain via control over hyperglycemia as well as hyperlipidemia to down-regulate generation of free radical, release of pro-inflammatory cytokines as well as elevation in membrane bound enzyme.(9)

According to another previous research by Manikandan et al studying the Anticataractogenic effect of hesperidin in galactose-induced cataractogenesis in Wistar rats it was observed that Galactose enriched food produced cataract in both the eye lens as a sequel to elevated serum glucose.(10) Simultaneous administration of hesperidin not only reduced serum glucose but also prevented cataract development, through reduced levels of reactive oxygen species (NO and OH) and iNOS expression as well as elevated enzymic and non-enzymic antioxidants were observed in the eye lens.(11)

CONCLUSION

In summary, while preliminary studies suggest that hesperidin may have modulatory effects on sorbitol accumulation and AGE formation, further research is necessary to elucidate the underlying mechanisms and evaluate the clinical relevance of these findings.It is important to note that the available research on hesperidin's modulatory activities on sorbitol accumulation and AGEs is still limited and primarily based on preclinical studies. The specific mechanisms of action and the clinical relevance of hesperidin in human populations require further investigation through well-designed clinical trials.

CONFLICT OF INTEREST:

The authors report no conflict of interest. The authors alone are responsible for the content and detailing of the paper.

SOURCE OF FUNDING:

Apoorva dental healthcare and oral rehabilitation centre

ETHICAL CLEARANCE: Since it is an in vitro study, ethical clearance is not required.

AUTHOR CONTRIBUTION:

All authors are equally contributed.

ACKNOWLEDGEMENT:

We extend our sincere gratitude to the Saveetha Dental College and Hospital and Department Of Forensic Odontology for their constant support and successful completion of this work.

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