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EVALUATION OF ISORHAMNETIN FOR ITS INHIBITORY ACTIVITY ON AMYLASE AND ADVANCED GLYCATION END PRODUCTS

Anuska Balaji[1], Dr. Abirami Arthanari[2]* and P. Parameshwari[3]

[1]Undergraduate Student, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Science (SIMATS) Saveetha University, Chennai, India

[2]Senior Lecturer, Department of Forensic Odontology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Science (SIMATS) Saveetha University Chennai - 600077, India

Researcher, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Science (SIMATS) Saveetha University, Chennai - 600077, India

ABSTRACT

Introduction: *Diabetes mellitus is a chronic metabolic disorder affecting millions of people globally, characterised by hyperglycemia and the formation of Advanced Glycation End Products (AGEs). Aldose reductase, an enzyme involved in the polyol pathway, plays a crucial role in diabetic complications due to its involvement in sorbitol production and AGE formation. In recent years, natural compounds from plants have garnered attention for their potential to inhibit aldose reductase and reduce AGE formation.*

Isorhamnetin, a flavonoid abundant in various fruits and vegetables, has been reported to possess diverse biological activities, including antioxidant, anti-inflammatory, and anticancer properties.This study aims to evaluate the inhibitory activity of isorhamnetin on aldose reductase and its effects on the formation of AGEs.

Aim*: To evaluate Isorhamnetin for its inhibitory activity on amylase and advanced glycation end products.*

Materials and Methods: *Firstly, isorhamnetin will be isolated and purified from natural sources using validated extraction techniques, followed by structural characterization using spectroscopic methods such as UV-Vis, IR, and NMR spectroscopy.The inhibitory activity of isorhamnetin against aldose reductase will be assessed through enzymatic assays to determine the IC50 value and gain insights into the nature of inhibition.*

Results*: The results of this study will shed light on the inhibitory activity of isorhamnetin on aldose reductase and its ability to attenuate AGE formation. If the outcomes are promising, isorhamnetin could emerge as a natural therapeutic agent for managing diabetes and its complications, offering a potential adjunct to current treatment strategies.*

Conclusion*: This research contributes to the growing body of knowledge on natural compounds' potential in combating diabetic complications, ultimately improving the quality of life for individuals living with diabetes.*

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by elevated blood glucose levels due to impaired insulin secretion or insulin resistance. (1)It affects millions of people worldwide and poses a significant public health challenge.(1,2) One of the key pathophysiological features of diabetes is the formation of Advanced Glycation End Products (AGEs).(3) AGEs are a heterogeneous group of molecules that result from the non-enzymatic reaction between reducing sugars and proteins or lipids.(3) Their accumulation contributes to various diabetic complications, including nephropathy, neuropathy, retinopathy, and cardiovascular diseases.

Controlling postprandial hyperglycemia is crucial in managing diabetes, and one approach is through inhibiting carbohydrate-digesting enzymes like α-amylase, which plays a pivotal role in the hydrolysis of complex carbohydrates into simple sugars.(4)Natural compounds from plants have gained attention for their potential in inhibiting α-amylase and reducing postprandial hyperglycemia. One such compound is isorhamnetin, a flavonoid found abundantly in various fruits and vegetables.(5)

Isorhamnetin (3-methylquercetin) belongs to the flavonol subclass of flavonoids and has been reported to exhibit various biological activities, including antioxidant, anti-inflammatory, and anticancer properties.(6) Recently, there has been growing interest in investigating the potential of isorhamnetin as an inhibitor of α-amylase and its ability to mitigate AGE formation, which could make it a promising candidate for managing diabetes and its associated complications.(6,7)

In this study, we aim to comprehensively evaluate the inhibitory activity of isorhamnetin on α-amylase and its effects on the formation of AGEs.(6–8) The investigation involves conducting in vitro experiments to elucidate the mechanisms underlying isorhamnetin's potential as an antidiabetic agent.(9)

To begin, we will isolate and purify isorhamnetin from natural sources using validated extraction methods. Characterization of the purified compound will be carried out using various spectroscopic techniques, such as UV-Vis, IR, and NMR spectroscopy, to ensure its purity and structural identification. Next, the inhibitory activity of isorhamnetin against α-amylase will be evaluated using enzymatic assays.(10)By analysing the inhibition kinetics, we can gain insights into the nature of the inhibition, whether it is competitive, non-competitive, or mixed-type. Furthermore, we will determine the IC50 value to quantify the potency of isorhamnetin as an α-amylase inhibitor.(10,11)

The investigation will then extend to studying the effect of isorhamnetin on AGE formation. In this part of the study, protein and lipid glycation assays will be performed to assess the potential of isorhamnetin in reducing AGE accumulation. Moreover, the antioxidant properties of isorhamnetin will be assessed, as they play a crucial role in combating oxidative stress and AGE formation.(8)

To validate the in vitro findings, we will also explore the potential of isorhamnetin to lower postprandial hyperglycemia in animal models. The impact of isorhamnetin on glucose homeostasis will be investigated by performing glucose tolerance tests and measuring insulin levels in experimental animals(1,8). Finally, we will discuss the implications of our findings, emphasising the potential of isorhamnetin as a natural antidiabetic agent. If the results prove promising, isorhamnetin could pave the way for novel therapeutic interventions to manage diabetes and its complications effectively.(12)

This study aims to provide valuable insights into the inhibitory activity of isorhamnetin on α-amylase and its ability to attenuate AGE formation. The results obtained from this research could lay the foundation for the development of isorhamnetin-based interventions for diabetes management and offer a potential adjunct to current therapies for improving the quality of life for individuals living with diabetes.

MATERIALS AND METHODS

Chemicals and Reagents

Isorhamnetin, 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 Isorhamnetin 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 Isorhamnetin (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 90µL of DL-glyceraldehyde (25 mM in potassium buffer) were mixed with 9µL of different concentrations of Isorhamnetin (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.

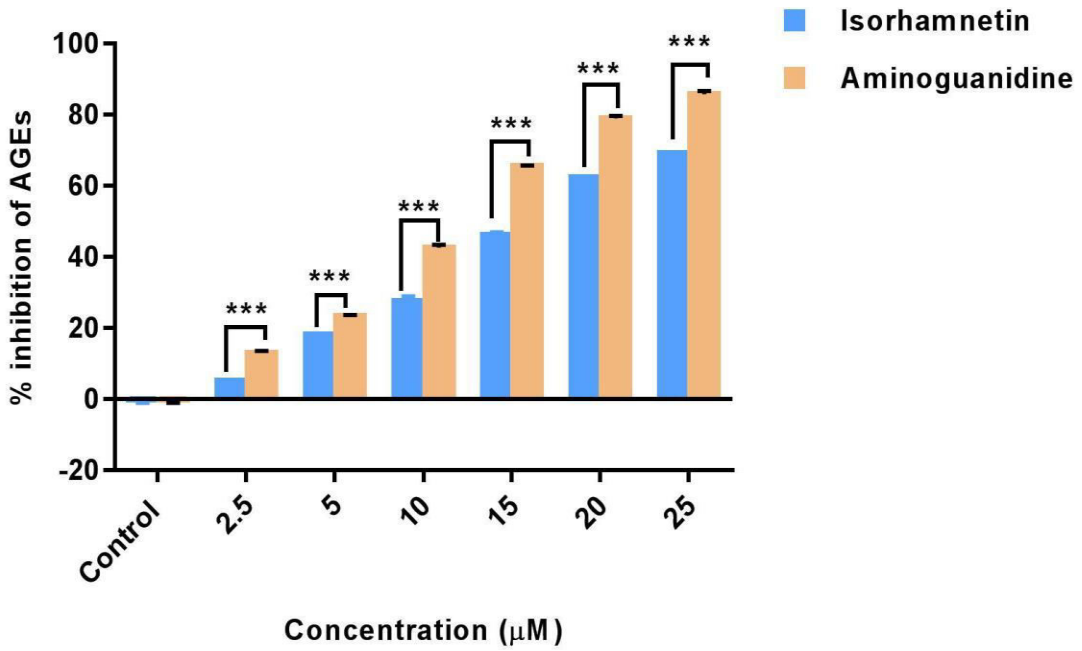
Sorbitol Accumulation Inhibition Assay (Malone et al., 1980)

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 Isorhamnetin on sorbitol accumulation was evaluated by incubating the RBC with different concentrations of the Isorhamnetin (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.

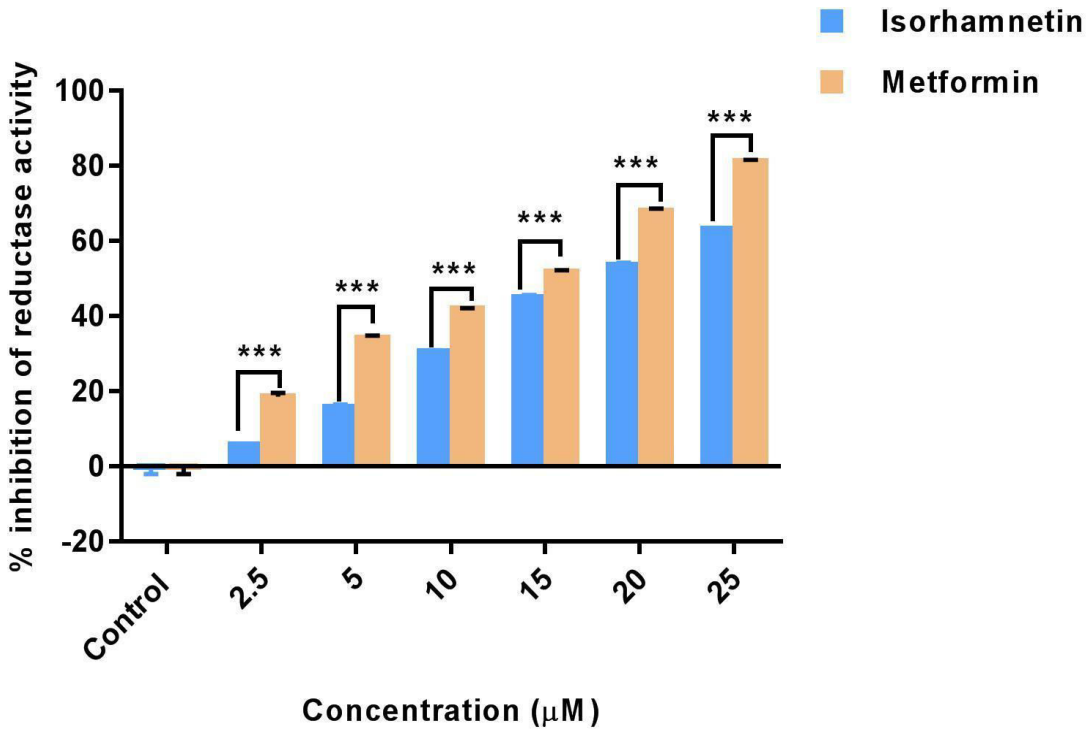
STATISTICAL ANALYSIS

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.

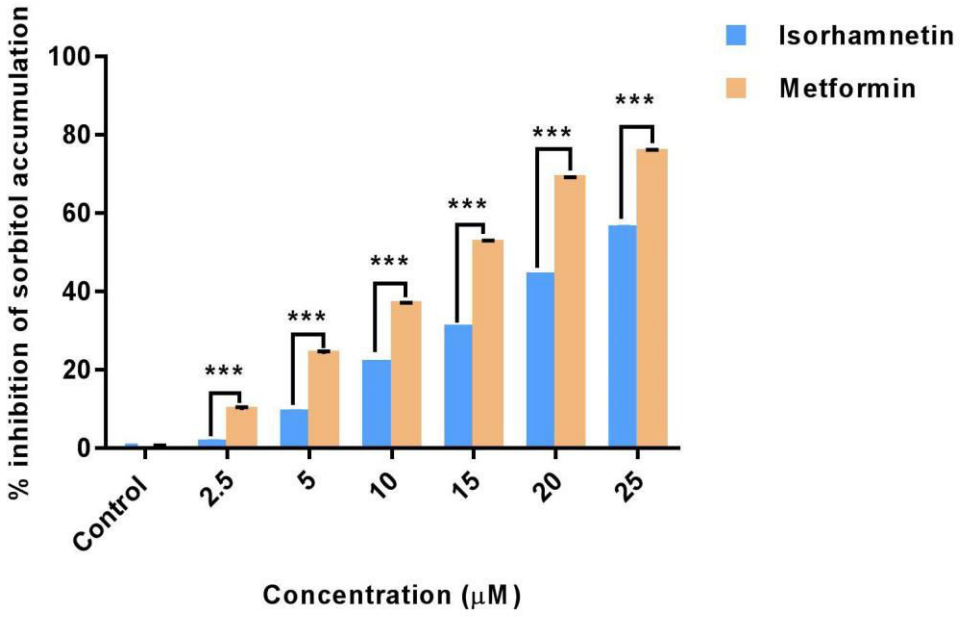
RESULTS



Graph 1



Graph 2



Graph 3

DISCUSSION

Isorhamnetin, a flavonoid compound present in various plants, has attracted significant attention for its potential therapeutic properties. Studies have investigated its inhibitory activity on amylase, an enzyme involved in carbohydrate digestion, as well as its effects on advanced glycation end products (AGEs). This discussion aims to explore the current state of research on isorhamnetin's inhibitory activity on amylase and its potential impact on AGE formation.(13)

1. Inhibitory Activity on Amylase:

1.1 Mechanism of Amylase Inhibition:

Studies have revealed that isorhamnetin can interact with amylase, leading to its inhibition. By preventing amylase from breaking down complex carbohydrates into simple sugars, isorhamnetin may delay or reduce the rate of glucose absorption in the small intestine. This property is of great interest in managing postprandial glucose levels and diabetes.

1.2 Efficacy in Diabetes Management:

Research on isorhamnetin's inhibitory activity on amylase has shown promising results in animal models and in vitro studies. However, there is a need for well-designed clinical trials to assess its efficacy and safety in humans. Understanding the dosage, bioavailability, and potential side effects is essential before considering isorhamnetin as a complementary therapy for diabetes management.

1.3 Potential Synergy with Other Anti-Diabetic Agents:

Combining isorhamnetin with existing anti-diabetic medications, such as alpha-glucosidase inhibitors or insulin sensitizers, may lead to synergistic effects in controlling blood glucose levels. Further investigations are warranted to explore these possibilities and ensure the safety and effectiveness of such combinations.(14)

2. Effects on Advanced Glycation End Products (AGEs):

2.1 Mechanism of AGE Formation Inhibition:

AGEs are formed when proteins or lipids undergo non-enzymatic glycation, leading to irreversible cross-linking and impaired cellular function. Isorhamnetin's antioxidative and anti-inflammatory properties have been proposed

to inhibit the formation of AGEs by reducing oxidative stress and inflammation, both of which contribute to glycation processes.

2.2 Implications for Age-Related Disorders:
Accumulation of AGEs has been associated with age-related diseases, including neurodegenerative disorders, cardiovascular diseases, and diabetic complications. Inhibition of AGE formation by isorhamnetin offers potential therapeutic implications in managing or preventing these disorders.

2.3 Considerations for Dosing and Administration:
The dosage and bioavailability of isorhamnetin are crucial factors when considering its potential as an anti-AGE agent. Studies are needed to identify the optimal dosage and the most effective route of administration to achieve the desired therapeutic effects.

3. Future Directions and Challenges:

3.1 Bioavailability and Metabolism Studies:
Understanding the bioavailability and metabolism of isorhamnetin is essential for determining its pharmacokinetics and ensuring its efficient delivery to target tissues. Formulation advancements, such as nanoencapsulation or prodrug development, may enhance its bioavailability and overall efficacy.

3.2 Safety and Toxicity Profile:
While isorhamnetin is generally considered safe in moderate quantities from dietary sources, high doses or long-term use may pose potential side effects. Comprehensive toxicological studies are necessary to determine its safety profile and establish appropriate dosage guidelines.

3.3 Human Clinical Trials:
The translation of preclinical findings into clinical trials is crucial to assess the efficacy and safety of isorhamnetin in humans. Well-designed randomized controlled trials are needed to validate its inhibitory activity on amylase and its potential to reduce AGE formation in relevant patient populations.

CONCLUSION
The evaluation of isorhamnetin for its inhibitory activity on amylase and advanced glycation end products holds immense promise in the management of diabetes and age-related disorders. While preclinical studies have demonstrated encouraging results, further research, including human clinical trials, is necessary to establish its safety, efficacy, and therapeutic potential. By addressing the challenges and limitations, researchers can pave the way for harnessing the full therapeutic potential of isorhamnetin for various health applications.

Author Contribution:
All author have equally contributed to the research.

Ethical CLearance Number:
Since it is an in vitro study,ethical clearance is not needed.

Conflict of Interest: Nil

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