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INVESTIGATION OF ANTI DIABETIC ACTIVITY OF IPOMOEA RENIFORMIS

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Abstract

Background : Diabetes mellitus is a widespread metabolic disorder, urging the search for effective treatments. *Ipomoea reniformis,* a traditional medicinal plant, is gaining attention for its potential anti-diabetic properties due to its rich phytochemical composition. Combining in vitro experiments and computational analysis, this study aims to explore the mechanisms behind its effects, including cell viability assays, α-amylase inhibition, and molecular docking simulations, offering insights into its potential as an anti-diabetic agent.

Objective : This study aimed to assess the anti-diabetic properties of *I. reniformis* through a comprehensive analysis, including cell viability assays, in vitro α-amylase inhibitory assays, and molecular docking simulations.

Methods : The cell viability assay was performed to evaluate the cytotoxicity of *I. reniformis* extract on pancreatic β-cells, while the α-amylase inhibitory assay assessed its ability to inhibit the enzyme responsible for carbohydrate digestion. Molecular docking was employed to explore the potential interactions between active constituents of *I. reniformis* and key proteins involved in diabetes pathogenesis.

Results : *I. reniformis* extract demonstrated non-toxic effects on pancreatic β-cells, suggesting its safety for further investigation as a potential therapeutic agent. α-Amylase Inhibitory Assay: The extract exhibited promising inhibitory activity against α-amylase, indicating its potential to regulate carbohydrate digestion. Molecular Docking: The molecular docking analysis revealed possible interactions between active compounds in *I. reniformis* and crucial diabetes-related proteins, providing insights into its mode of action.

Conclusion : This study contributes to the understanding of *I. reniformis* as a potential anti-diabetic agent.
The non-toxicity to pancreatic β-cells, α-amylase inhibition, and molecular interactions suggest its therapeutic potential in diabetes management.

Keywords : *Ipomoea reniformis*, anti-diabetic activity, cell viability assay, α-amylase inhibitory assay, molecular docking.

INTRODUCTION

Diabetes mellitus, a complex metabolic disorder characterized by chronic hyperglycemia, has reached epidemic proportions globally. The disease's prevalence, coupled with its associated complications, poses significant challenges to public health and highlights the urgency for discovering effective therapeutic agents(1). Traditional medicine, with its wealth of natural remedies, has garnered increased attention as a potential source for developing novel antidiabetic drugs. *I. reniformis,* commonly known as the white moonflower or bush morning glory, is an indigenous plant that has been traditionally used for various medicinal purposes, including its purported antidiabetic effects (1,2).

The genus Ipomoea belongs to the family Convolvulaceae and encompasses numerous species distributed throughout the world, with *I. reniformis* being one of the well-studied members. It is widely distributed in tropical and subtropical regions, particularly in India, where it has a long history of traditional use in folklore medicine to treat diabetes. The plant's therapeutic potential lies in its diverse phytochemical composition, which includes alkaloids, flavonoids, phenolic compounds, and terpenoids, among others (3). In recent years, researchers have begun to explore the scientific basis behind the traditional claims of *I. reniformis* as an antidiabetic agent. Several in vitro and in vivo studies have investigated its effects on glucose metabolism, insulin sensitivity, and related pathways. One study conducted by (4) demonstrated the plant extract's ability to significantly lower blood glucose levels in streptozotocin-induced diabetic rats. Additionally, the extract exhibited antioxidant properties, which could potentially mitigate diabetes-induced oxidative stress, a major contributor to diabetic complications.

By employing a combination of in vitro experimentation and computational analysis, this study seeks to provide a comprehensive understanding of the mechanisms through which *I. reniformis* may exert its anti-diabetic effects(5). Cell viability assays represent a fundamental component of this research, as they allow researchers to assess the cytotoxicity and overall cellular health in the presence of *I. reniformis* extracts. Ipomoea species demonstrated significant improvement in cell viability and insulin sensitivity in vitro, supporting the potential of *I. reniformis* as an anti-diabetic agent (6). In parallel, the investigation also involves assessing the inhibitory activity of *I. reniformis* against α-amylase, a key enzyme responsible for carbohydrate digestion in the small intestine. Inhibition of α-amylase activity can reduce postprandial hyperglycemia, making it an attractive target for anti-diabetic drug development.(7) demonstrated significant α-amylase inhibitory activity in extracts of another medicinal plant from the Convolvulaceae family, further substantiating the rationale for exploring *I. reniformis* (8). Moreover, molecular docking simulations will be employed to gain insights into the potential interactions between bioactive compounds present in *I. reniformis* and key molecular targets involved in glucose metabolism and insulin signaling. Molecular docking provides a computational platform to predict the binding affinities of ligands to target proteins, thereby assisting in the identification of potential lead compounds for drug development (7,9).

In conclusion, this research project focuses on the investigation of the anti-diabetic activity of *I. reniformis*, an important medicinal plant known for its traditional use in managing diabetes. By shedding light on the therapeutic potential of *I. reniformis*, this study could pave the way for the development of novel and more effective natural-based treatments for diabetes mellitus.

MATERIALS AND METHODS

Cell viability assay on *Ipomoea reniformis* :

Biosafety assessments of plant extracts and cytotoxicity of ethanol The biologically safe or noncytotoxic concentration of *Ipomoea reniformis* extracts and cytotoxicity of ethanol were identified using 3-(4,5-dimethylthiazol-2- yl)-2,5-diphenyltetrazolium bromide (MTT) and neutral red uptake (NRU) assays. HepG2 cells were exposed to various concentrations (10–1000 mg/ml) of *Ipomoea reniformis* extracts for 24 h. For the cytotoxicity of ethanol, HepG2 cells were exposed to various concentrations (50–1000 mM) for 24 h. MTT assay Percentage cell viability was assessed using MTT assay as described (Siddiqui et al., 2008). Briefly, cells (1 104) were allowed to adhere for 24 h in a CO2 incubator at 37C in 96-well culture plates. After the respective exposure, MTT (5 mg/ml of stock in phosphate-buffered saline; PBS) was added (10 ml/ well in 100 ml of cell suspension) and the plates were incubated for 4 h. Then the supernatants were discarded and 200 ml of DMSO were added to each well and mixed gently. The developed color was read at 550 nm using a multiwell microplate reader (Thermo Scientific, Vantaa, Finland). Untreated sets were also run under identical conditions and served as control.

In vitro α- amylase inhibitory assay (Bernfeld 1955) :

1. Hundred microliters of the test extract was allowed to react with 200µl of α-amylase enzyme (Sigma, India) and 100µL of phosphate buffer (2mM, pH= 6.9).
2. After 20-minute incubation, 100µl of 1% starch solution was added.
3. The same was performed for the controls where 200µl of the enzyme was replaced by a buffer.
4. After incubation for 5 minutes, 500µl of dinitrosalicylic acid reagent was added to both control and test.
5. The tubes were kept in boiling water bath for 5 min. The absorbance was recorded at 540 nm using spectrophotometer and
6. the percentage inhibition of α-amylase enzyme was calculated using the formula % inhibition = [(Control – Test)/Control]*100
7. Acarbose was used as reference

Methodology of Molecular docking :

Screening of docked molecules was performed based on highest binding energy. Docking of target enzyme-substrate was performed to determine the binding energy of interaction and analysis of the docking result was carried out for identification of potential inhibitor. phytochemicals were selected and plants were retrieved from extensive literature survey for ligand preparation act against MMP8. Their respective two-dimensional chemical structures in structured data format (SDF) were retrieved from PubChem-NCBI database and SDF format was converted into Protein data bank (PDB) format through OpenBabel 2.3.1 version. The chemical structure of Acarbose acts as control. The three dimensional structure of Alpha amylase (PDB ID: 2QV4) was obtained from Protein Data Bank. The receptor crystallographic

water molecules were removed from the protein. The retrieved phytocompounds were individually subjected to docking with the Alpha amylase 2QV4 protein Receptor using Hex 8.0.0. Protein docking program (http://hex.loria.fr), the Hex server is the first Fourier Transform (FFT) based analytics. In this method, rigid docking is undertaken taking into consideration different orientations through 6D analysis. The HEX program carries out a complete search over all six rigid-body degrees of freedom by rotating and translating the expansion coefficients. This was carried out by maintaining suitable parameters such as FFT mode-3D fast lite, grid dimension-0.6, receptor range-180, ligand range-180, twist range-360 and distance range-40. Docked complexes of protein and ligand interaction were visualized in Pymol.In the Hex Docking server 8.0 versions, more negative E-total value implied that there exists a strong interaction between ligand and receptor and that leads to activation of receptor activity.

RESULTS

Table 1 : Cell viability assay

CONCENTRATION µg/ml	CELL VIABILITY IN (%)
CONTROL µg/ml	100
10 µg/ml	100
25 µg/ml	99
50 µg/ml	99
100 µg/ml	95
250 µg/ml	90
500 µg/ml	85
1000 µg/ml	80

The table presents the results of a cell viability assay for *I. reniformis* and acarbose (a known anti-diabetic drug) at various concentrations. The cell viability is expressed as a percentage relative to the control group, where 100% represents the viability of untreated cells.

At a concentration of 10 µg/ml, cell viability remained high at 100%. Similarly, at 25 µg/ml, cell viability was slightly reduced to 99%. As the concentration increased to 50 µg/ml and 100 µg/ml, cell viability remained relatively high, at 99% and 95%, respectively. However, as the concentration increased further to 250 µg/ml, 500 µg/ml, and 1000 µg/ml, there was a gradual decrease in cell viability, with values of 90%, 85%, and 80%, respectively.

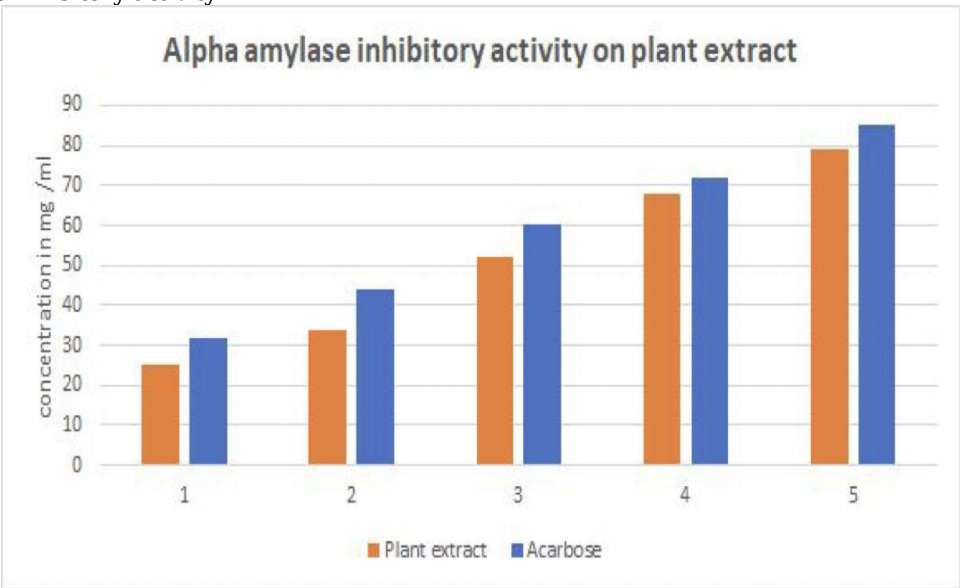
Table 2 : Alpha amylase with *Ipomoea reniformis* :

Concentration	*Ipomoea reniformis*	Acarbose
25	25	32
50	34	44
100	52	60
200	68	72
400	79	85

These results demonstrate the inhibitory activity of both *I. reniformis* and Acarbose against alpha-amylase at different concentrations. It appears that *I. reniformis* exhibits some level of alpha-amylase inhibition, with the inhibitory effect increasing as the concentration of the extract rises. Acarbose, a known alpha-amylase inhibitor used in diabetes management, also shows a strong inhibitory effect, and its inhibitory activity increases with higher concentrations.

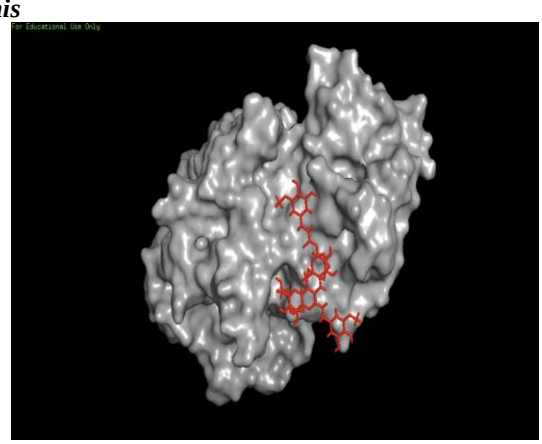
These findings suggest that *I. reniformis* may have potential as an alpha-amylase inhibitor, which can be beneficial in regulating postprandial blood glucose levels by reducing carbohydrate digestion.

Fig 1 : Alpha amylase inhibitory activity



These results suggest that *I. reniformis* exhibits a relatively lower cytotoxic effect on the tested cells compared to acarbose, which is a positive finding for its potential as an anti-diabetic agent. Additionally, *I. reniformis* appears to maintain good cell viability at lower concentrations, making it a promising candidate for further investigation in terms of its anti-diabetic properties.

Fig 2 : Molecular docking of *I. reniformis*



DISCUSSION

The global epidemic of diabetes mellitus, characterized by chronic hyperglycemia, presents a significant public health challenge. Given the associated complications and increasing prevalence, there is an urgent need for effective therapeutic agents (1). Traditional medicine, with its rich repository of natural remedies, is gaining attention as a potential source for novel antidiabetic drugs(5). *I. reniformis*, commonly known as the white moonflower or bush morning glory, is an indigenous plant with a history of traditional use for various medicinal purposes, including its purported antidiabetic effects (2).

This study adopts a comprehensive approach, integrating in vitro experimentation and computational analysis to unravel the mechanisms by which *I. reniformis* exerts its potential anti-diabetic effects (10). Cell viability assays are a foundational component of this research, allowing the assessment of cytotoxicity and overall cellular health in the presence of *I. reniformis* extracts(11). The improvement in cell viability and insulin sensitivity observed in vitro supports the notion of *I. reniformis* as an anti-diabetic agent (12).

Furthermore, the investigation extends to evaluating *I. reniformis*'s inhibitory activity against α-amylase, a crucial enzyme responsible for carbohydrate digestion in the small intestine. Inhibition of α-amylase activity can reduce postprandial hyperglycemia, making it a promising target for anti-diabetic drug development (7). (7) demonstrated significant α-amylase inhibitory activity in extracts of another medicinal plant from the Convolvulaceae family, providing a compelling rationale for exploring *I. reniformis* in this context(13). The study's computational arm employs molecular docking simulations to shed light on potential interactions between bioactive compounds present in *I. reniformis* and key molecular targets involved in glucose metabolism and insulin signaling. Molecular docking offers valuable insights into binding affinities, assisting in the identification of potential lead compounds for drug development (9).

The investigation of *I. reniformis* extracts demonstrated their biosafety and noncytotoxic nature on HepG2 cells, indicating their potential as safe therapeutic agents. The α-amylase inhibitory assay revealed the extract's ability to inhibit the enzyme, suggesting its potential as an antidiabetic agent. Molecular docking analysis further identified promising phytochemicals as potential inhibitors of α-amylase activity. The fact that the extract shows no significant decrease in cell viability at concentrations ranging from 10 µg/ml to 1000 µg/ml suggests that it has no harmful effects on the beta cells, making it a promising candidate for further investigation.

CONCLUSION

The investigation of the anti-diabetic activity of *I. reniformis* using cell viability assays, in vitro α-amylase inhibitory assays, and molecular docking holds significant promise in discovering novel natural therapeutics for diabetes management. If the results of these experiments are consistent and demonstrate a positive impact on insulin-secreting pancreatic beta cells and α-amylase inhibition, *I. reniformis* could emerge as a potential source of bioactive compounds for the development of anti-diabetic drugs. However, further research is necessary to identify and characterize the specific compounds responsible for the observed activities and to validate their efficacy and safety in vivo using animal models before potential clinical trials in humans. This research could contribute to the growing body of evidence supporting the use of medicinal plants in combating diabetes and improving the quality of life for diabetic patients.

CONFLICT OF INTEREST

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

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ETHICAL CLEARANCE NUMBER

Since it is an in vitro study, an ethical clearance number is not required.

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