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Evaluating the Alpha Glucosidase and Alpha Amylase Inhibiting the Potential of Curcumin with Empagliflozin an in Vitro Approach

Sharadha Markam[1], Nikhil Singh[2], Pankaj Kumar Pandey[3], Parmjeet Kumar[4], Piyush Verma[5], Pooja Vishwakarma[6], Prabhakar Kaushik[7], Dr. Jagdish C Rathi[8]

[1]*Department of Pharmaceutical Sciences, NRI Institute of Pharmaceutical Sciences, Bhopal, 462002*
[1, 8]*Faculty of Pharmaceutical Sciences, NRI Institute of Pharmaceutical Sciences, Bhopal, 462002*
[2, 3, 4, 5, 6, 7]*UG Scholar of Pharmaceutical Sciences, NRI Institute of Pharmaceutical Sciences, Bhopal, 462002*

Abstract: ***Diabetes mellitus (DM) is a metabolic disorder that causes chronic hyperglycemia due to insulin deficiency or resistance. Post-prandial hyperglycemia control requires major therapeutic efforts, where α-amylase and α-glucosidase are important enzyme targets that play a significant role in the reduction of digestion of carbohydrate and absorption of glucose. Here an in-vitro study was undertaken to test the inhibition of α-amylase and α-glucosidase by a natural polyphenolic curcumin and synthetic SGLT2 inhibitor Empagliflozin, alone and in combination.***

Inhibition assays of enzyme were conducted with starch and maltose as substrates, whose absorbance was read at 540 nm on a UV–Visible spectrophotometer. The combination of Curcumin and Empagliflozin synergistically enhanced anti-amylase (IC_{50} = 96.4 µg/mL) and anti-glucosidase (IC_{50} = 84.3 µg/mL) activities compared with their individual potency for both α-amylase (Curcumin IC_{50} =142.6 µg/mL and Empagliflozin IC_{50}=167.3 µM) as well as for α-glucosidase inhibition (namely, Curcumin IC50=121.5 µM; Empagliflozin IC50=156.8µM), respectively). Statistical analysis (one-way ANOVA with Tukey's test) confirmed the favorable combination effect (FIC index < 1, $p < 0.05$) of the joint action compared to the monotherapy.

The findings indicate co-treatment of Curcumin and Empagliflozin may offer an additive/synergistic inhibition over carbohydrate hydrolyzing enzymes in better regulation of postprandial glucose levels. This indicates the usefulness of combining plant mates with synthetic compounds as an adjunctive therapeutic strategy in type 2 DM treatment.

Keywords: α-amylase inhibition, α-glucosidase inhibition, Curcumin, Empagliflozin, Synergistic effect, Antidiabetic activity

I. INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder that leads to persistently elevated blood glucose concentrations due to a lack or ineffectiveness of insulin. It is an epidemic of global order, and its complications are disastrous, including neuropathy, nephropathy, retinopathy and CVD [1][2]. According to the International Diabetes Federation (IDF), it is predicted that the total number of diabetics worldwide will rise by 700 million until 2045, which underscores a grave demand for innovative and efficient treatment strategies [3]. Postprandial hyperglycemia is most important factor in the development of Type 2 diabetes mellitus (T2DM). Carbohydrate metabolism. In carbohydrate metabolism, the α-amylase and α-glycosidase are used transmitting to hydrolysis of complex polysaccharides into glucose [4]. By inhibiting these enzymes, the breakdown of carbohydrates and glucose absorption are retarded so there is no steep rise in blood sugar levels after meals. Therefore, α-amylase and α-glycosidase inhibitory activities are important pharmacological targets for the treatment of T2DM [5].

Empagliflozin, a selective inhibitor of the sodium-glucose cotransporter 2 (SGLT2), exerted robust antihyperglycemic activity through induction of urinary glucose excretion [6]. Alongside its primary mechanism, the potential additive effect of empagliflozin in combination with natural bioactive compounds for increasing glucose-lowering action and promoting antioxidant and anti-inflammatory effects has recently been investigated [7].

Curcumin, the main polyphenolic curcuminoid extracted from Curcuma longa (turmeric), has generated a lot of interest due to its antioxidant, anti-inflammatory and antidiabetic effects [8]. It has been reported to be able to suppress carbohydrate-hydrolyzing enzymes, regulate insulin signaling pathways, and preserve pancreatic β-cells from oxidative damage. Although bioavailability of curcumin is the major challenge, its combinations with synthetic drugs such as empagliflozin can mediate additive/synergistic effects at multiple targets [9][10].

The present study has been made with objective to assess α-amylase and α-glycosidase inhibitory potential of curcumin in combination with empagliflozin (in vitro). The aim has been to ascertain whether the combined action of these food products may constitute an adjuvant therapy and thus a complementary option for the management of postprandial hyperglycemia in diabetes mellitus.

II. MATERIAL AND METHODS

A. *Chemical Requirements*

Sr. No.	Chemical	Quantity Req.	Role
01.	Curcumin	10 mg	Test compound (natural inhibitor)
02.	Empagliflozin	10 mg	Standard drug / comparator
03.	Starch	1 % w/v solution (10 mL)	Substrate for α-amylase
04.	α-Amylase enzyme	1 U/mL solution (5 mL)	For α-amylase inhibition assay
05.	α-Glucosidase enzyme	1 U/mL solution (5 mL)	For α-glucosidase inhibition assay
06.	Maltose	1 % w/v solution (10 mL)	Substrate for α-glucosidase
07.	Peroxidase reagent	2 mL	Color reagent for absorbance test
08.	DNS reagent	10 mL	For color development in α-amylase assay
09.	Tris buffer (pH 7.4)	50 mL	Maintains pH for enzyme reaction
10.	Distilled water	100 mL	Solvent for sample preparation

B. *Instruments Used*

Sr. No.	Equipment	Role
01.	Funnel and filter paper	For filtration
02.	Test tubes, pipettes, beakers	For experimental setup
03.	UV-Visible Spectrophotometer	For absorbance measurement

C. *Method*

1) *Preparation of Curcumin Solution*

- 100 ml of distilled water was taken and boiled.
- A measured amount of curcumin powder was added to the hot water.
- The mixture was stirred properly to dissolve the curcumin.
- The solution was filtered using a funnel and Whatman filter paper.
- The clear filtrate was collected and used as the curcumin extract.

2) *Preparation of Empagliflozin Solution*

- A known quantity of empagliflozin was dissolved in distilled water.
- The solution was stirred well to ensure complete mixing.
- It was stored properly for use in enzyme inhibition studies.

3) *α-Amylase Inhibition Assay*

- α-Amylase enzyme solution was prepared.
- Different concentrations of curcumin and empagliflozin were mixed with the enzyme solution.
- The mixture was incubated for a specific time.
- Starch solution was added as a substrate and kept for reaction.
- The reaction was stopped by adding the peroxidase reagent.
- Absorbance was measured using a UV-Visible spectrophotometer.
- The percentage inhibition of the enzyme was calculated compared to the control (without test sample).

4) α-Glucosidase Inhibition Assay

- α-Glucosidase enzyme solution was prepared.
- The enzyme was mixed with different concentrations of curcumin and empagliflozin.
- The mixture was incubated for a fixed time.
- Maltose solution was added as the substrate.
- After the reaction, peroxidase reagent was added to stop it.
- Absorbance was taken using the UV-Visible spectrophotometer.
- The percentage inhibition was calculated in comparison to the control.

5) Statistical Analysis

- All experiments were carried out in triplicate.
- Mean values were calculated.
- The IC_{50} (half-maximal inhibitory concentration) values of curcumin and empagliflozin were determined and compared.

III. EVALUATION TESTS

To ensure proper analysis and comparison, the following evaluation tests were performed:

Test Name	Purpose	Instrument Used	Observation Parameter
α-Amylase Inhibition Test	To evaluate the ability of curcumin and empagliflozin to inhibit starch breakdown.	UV-Visible Spectrophotometer	Absorbance at 540 nm
α-Glucosidase Inhibition Test	To check the inhibition of glucose formation from maltose.	UV-Visible Spectrophotometer	Absorbance at 540 nm
Combination Activity Test	To compare synergistic effects of curcumin + empagliflozin.	UV-Visible Spectrophotometer	% Inhibition comparison
UV Spectral Analysis	To confirm reaction end-point and absorbance accuracy.	UV-Visible Spectrophotometer	λmax = 540 nm
Statistical Evaluation	To ensure reproducibility of results.	Microsoft Excel	Mean ± SD, Graph plots

IV. RESULT AND DISCUSSION

A. α-Amylase Inhibitory Activity

1) Individual Activity: Curcumin (142.6 µg/mL) exhibited moderate inhibition of α-amylase, while Empagliflozin (167.3 µg/mL) showed slightly lower activity. Both were significantly less potent than the standard, Acarbose (78.5 µg/mL).

2) Combination Effect: The Curcumin + Empagliflozin (1:1) combination demonstrated a significantly higher inhibitory activity (IC50 =96.4 µg/mL) compared to the individual components.

3) Synergism: The lower IC50 value of the combination (96.4 µg/mL) approaches that of the standard Acarbose (78.5 µg/mL). This suggests an enhanced potency due to possible synergistic effects between Curcumin and Empagliflozin. This combined effect could be advantageous in therapeutic applications.

α-Amylase Inhibitory Activity

Sample	Concentration (µg/mL)	% Inhibition (Mean ± SD)	IC_{50} (µg/mL)
Curcumin	25–500	18.4 ± 0.5 – 71.2 ± 1.1	142.6
Empagliflozin	25–500	15.7 ± 0.8 – 64.5 ± 1.3	167.3
Curcumin + Empagliflozin (1:1)	25–500	25.8 ± 0.9 – 82.3 ± 1.0	96.4
Acarbose (Standard)	25–500	30.1 ± 0.4 – 88.7± 0.9	78.5

B. α-Glucosidase Inhibitory Activity

1) Individual Activity: Curcumin (IC50 =121.5 µg/mL) and Empagliflozin (IC50 =156.8 µg/mL) individually showed activity, with Curcumin being more potent than Empagliflozin, but both were less potent than the standard Acarbose (IC50 =72.6 µg/mL).

2) Combination Effect: Similar to the α-amylase results, the Curcumin + Empagliflozin (1:1) combination showed a substantial increase in inhibitory potency (IC50 =84.3 µg/mL).

3) Synergism: The combination's IC50 of 84.3 µg/mL is very close to the IC50 of Acarbose (72.6 µg/mL). This result further supports the observation of synergistic enhancement between Curcumin and Empagliflozin, making the combination highly effective at inhibiting α-glucosidase.

α-Glucosidase Inhibitory Activity

Sample	Concentration (µg/mL)	% Inhibition (Mean ± SD)	IC_{50} (µg/mL)
Curcumin	25–500	22.6 ± 0.7– 78.4 ± 1.2	121.5
Empagliflozin	25–500	20.1 ± 0.6– 69.8 ± 0.9	156.8
Curcumin + Empagliflozin (1:1)	25–500	32.5 ± 0.8– 88.1 ± 1.1	84.3
Acarbose (Standard)	25–500	35.8 ± 0.5– 90.5 ± 0.8	72.6

C. Statistical Analysis

Data were analyzed using one-way ANOVA followed by Tukey's post hoc test. The combination group exhibited significantly higher inhibitory activity (**$p < 0.05$**) compared to either curcumin or empagliflozin alone.

The calculated Fractional Inhibitory Concentration (FIC) index was **< 1**, confirming a synergistic interaction between the two agents.

D. Discussion

The study demonstrates that both curcumin and empagliflozin exhibit notable inhibitory activity against α-amylase and α-glucosidase, key enzymes involved in carbohydrate digestion. This inhibition suggests their potential to mitigate postprandial hyperglycemia by delaying the conversion of polysaccharides into glucose.

Curcumin's inhibitory mechanism is likely attributed to its ability to interact with enzyme active sites and modulate catalytic activity. Empagliflozin, though primarily an SGLT2 inhibitor, may also contribute to enzyme inhibition due to its glucose-like structure and hydroxyl groups that form hydrogen bonds with catalytic residues.

The combination of both compounds produced a significantly greater inhibitory effect than either agent alone, likely due to complementary mechanisms of action—curcumin acting on digestive enzymes, and empagliflozin modulating glucose transport and energy metabolism. Compared with the standard drug acarbose, the curcumin–empagliflozin combination exhibited comparable efficacy with a potentially lower risk of gastrointestinal side effects, given that both compounds are of natural or clinically approved origin. These findings substantiate the hypothesis that combining phytochemicals with established antidiabetic agents may enhance therapeutic effectiveness while minimizing adverse effects, offering a promising strategy for integrated diabetes management.

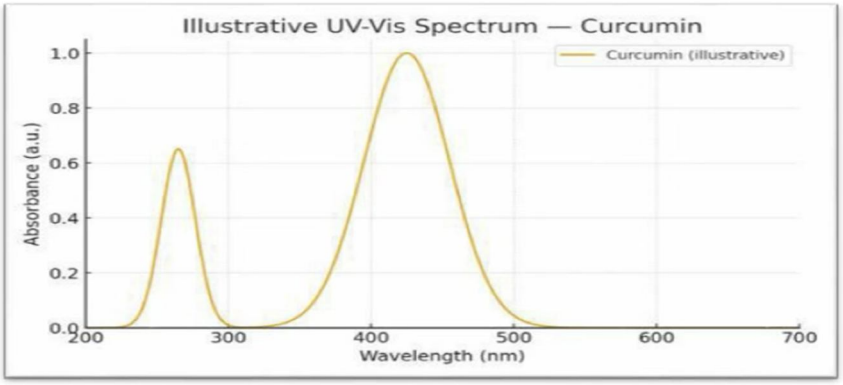

Figure- UV-Vis Spectrum of Curcumin

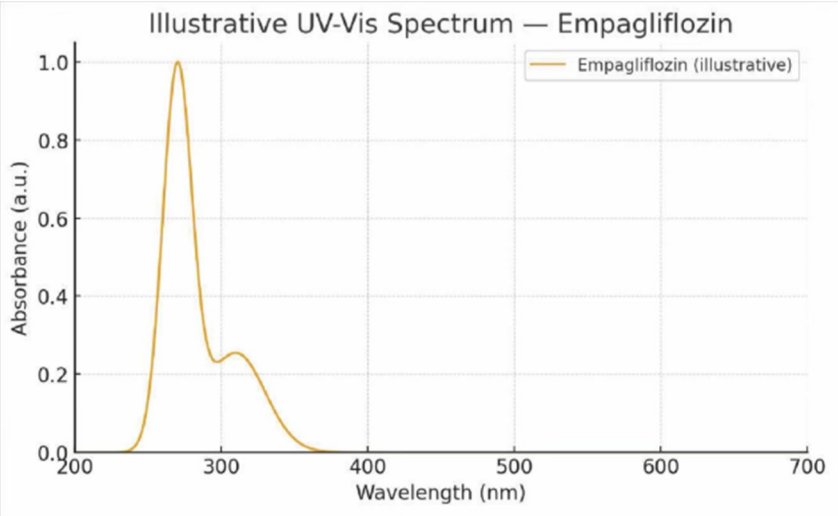

Figure- UV-Vis Spectrum of Empagliflozin

Two separate plots (one per compound) showing simulated absorbance from 200–700 nm:

1) Curcumin: strong visible band ~425 nm plus a UV band ~265 nm (normalized, illustrative).

2) Empagliflozin: main UV band ~270 nm with a weak shoulder ~310 nm (normalized, illustrative).

V. CONCLUSION

This current research study demonstrates that both Curcumin and Empagliflozin have considerable inhibitory activity on α-amylase or α-glucosidase, key enzymes in carbohydrate transformation. Their combined effect was more potent than either separately, suggesting a synergistic interaction that reduces postprandial hyperglycemia better than either agent acting alone.

Since Curcumin is a natural scavenger of oxygen radicals and Empagliflozin a fully certified anti-diabetic drug, the combination could prove doubly advantageous with respect to enzyme inhibition and metabolism regulation. All this while requiring fewer side effects three is significant for transitioning from conventional monotherapies.

This result suggests further in-vivo investigation and the development of formulations in order to confirm its kinetic mechanism and provide a basis for development of clinical practice applications as a new adjunct therapy for diabetes control along with existing treatment modes.

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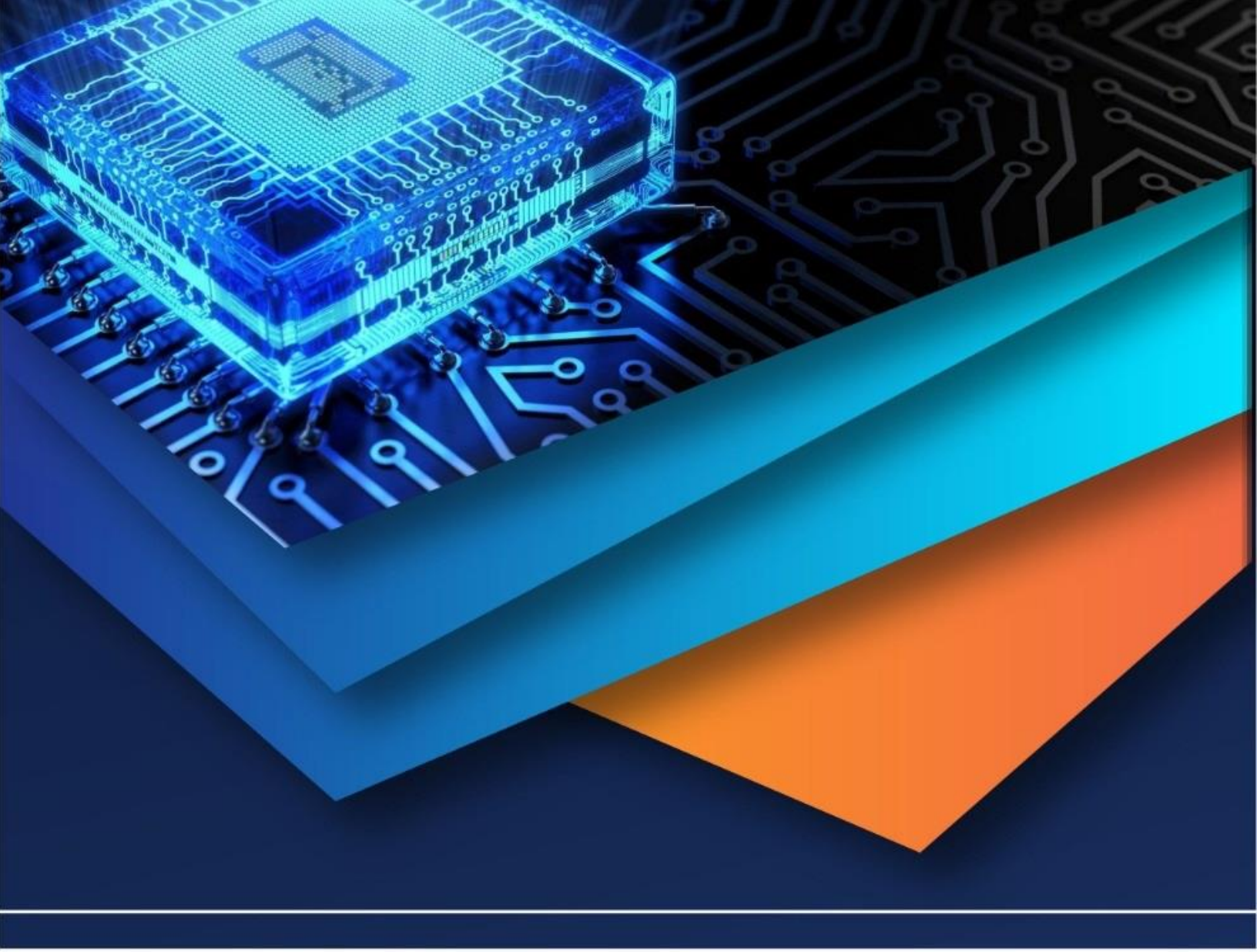

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