In Vitro Evaluation of Standardized White Mulberry Leaf Extract for Postprandial Glucose Control: Dual Mechanisms of Enzyme Inhibition and Insulin-Sensitizing Activity.

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Abstract

This study systematically evaluated the antidiabetic potential of a standardized hydro-ethanolic extract of *Morus alba L.* leaves (White Mulberry Leaf Extract, WMLE). The extract was standardized to contain 1.82% 1-deoxynojirimycin (DNJ) and 3.05% rutin. WMLE exhibited potent, dose-dependent inhibition of α-glucosidase (IC_{50} = 38.5 µg/mL) and α-amylase (IC_{50} = 245.7 µg/mL), with preferential activity against α-glucosidase. The extract also significantly stimulated glucose uptake in L6 skeletal muscle cells, both independently and synergistically with insulin. Synergy analysis revealed enhanced efficacy of the whole extract compared to isolated fractions (Combination Index = 0.78). These findings suggest WMLE acts through dual mechanisms—slowing carbohydrate digestion and enhancing peripheral glucose disposal—making it a promising multi-target botanical for managing postprandial hyperglycemia and insulin resistance in type 2 diabetes.

Keywords: *Morus alba*, White Mulberry, α-glucosidase inhibition, postprandial hyperglycemia, diabetes, 1-deoxynojirimycin, insulin sensitivity, synergy.

1. Introduction

Diabetes mellitus (DM) is a global health crisis, with type 2 diabetes (T2DM) accounting for 90–95% of cases[1]. Postprandial hyperglycemia (PPH) is a key contributor to diabetic complications, driving oxidative stress, endothelial dysfunction, and vascular damage[2]. While alpha-glucosidase inhibitors (AGIs) like acarbose effectively reduce PPH, their use is limited by gastrointestinal side effects[3]. This has spurred interest in natural AGIs with improved tolerability.

White Mulberry (*Morus alba L.*) has a long history of use in traditional medicine for diabetes ("Xiao Ke" syndrome)[4]. Its leaves contain 1-deoxynojirimycin (DNJ), a potent iminosugar inhibitor of α-glucosidase and α-amylase, along with flavonoids and phenolic acids that may enhance insulin sensitivity and provide antioxidant benefits[56]. However, systematic in vitro comparisons with standard drugs and investigations into the temporal kinetics and synergistic interactions of its constituents are limited.

This study aimed to evaluate the in vitro efficacy of a standardized WMLE, compare its potency to acarbose, assess its time-dependent enzyme inhibition, and explore its effects on glucose uptake in muscle cells.

2. Objectives

Primary Objectives:

1. To prepare and standardize a hydro-ethanolic WMLE containing ≥1% DNJ.

2. To determine the IC_{50} of WMLE against α-glucosidase and α-amylase.

3. To compare WMLE's α-glucosidase inhibitory potency with acarbose.

Secondary Objectives:

4. To investigate the time-dependent inhibition profile of WMLE.

5. To assess WMLE's effect on glucose uptake in L6 skeletal muscle cells.

6. To analyze phytochemical interactions within WMLE.

3. Materials and Methods

3.1. Chemicals and Reagents

α-Glucosidase (from *S. cerevisiae*), α-amylase (porcine pancreas), p-nitrophenyl-α-D-glucopyranoside (pNPG), soluble starch, acarbose, and all solvents were obtained from Sigma-Aldrich and HiMedia. All chemicals were analytical grade.

3.2. Preparation and Standardization of WMLE

Dried *Morus alba* leaves were extracted with 70% ethanol. The extract was concentrated and dried (yield: 18.7% w/w). DNJ and rutin content were quantified using validated HPLC-ELSD and HPLC-DAD methods, respectively.

3.3. α-Glucosidase Inhibition Assay

The assay used pNPG as substrate[7]. Enzyme and inhibitor were pre-incubated (37°C, 10 min), followed by substrate addition and incubation (30 min). Reaction was stopped with Na_2CO_3, and absorbance read at 405 nm. Percent inhibition and IC_{50} were calculated.

3.4. α-Amylase Inhibition Assay

The DNSA method was used[8]. Starch solution, enzyme, and inhibitor were incubated (37°C, 15 min). Reaction stopped with HCl, followed by DNSA reagent addition and boiling. Absorbance was measured at 540 nm.

3.5. Glucose Uptake Assay in L6 Myotubes

Differentiated L6 skeletal muscle cells were treated with WMLE (10–100 µg/mL) with/without insulin (100 nM). Glucose uptake was measured using 2-deoxyglucose uptake assay[9].

3.6. Synergy Analysis

WMLE was fractionated into aqueous (AQF) and ethyl acetate (EAF) fractions. Fractions and their reconstituted mixture were tested for α-glucosidase inhibition. Combination Index (CI) was calculated using the Chou-Talalay method[10].

3.7. Statistical Analysis

All assays were performed in triplicate. Data expressed as mean ± SD. IC_{50} values determined via nonlinear regression. Comparisons used unpaired t-test or ANOVA with Tukey's post-hoc test ($p < 0.05$ significant).

4. Results

4.1. Phytochemical Standardization

WMLE yielded 18.7% w/w. HPLC analysis confirmed DNJ content of 1.82 ± 0.14% w/w and rutin content of 3.05 ± 0.21% w/w.

Table 1: Standardization Parameters of WMLE

Parameter	Method	Marker Compound	Content (% w/w, Mean ± SD)
Iminosugar	HPLC-ELSD	DNJ	1.82 ± 0.14
Flavonoid	HPLC-DAD	Rutin	3.05 ± 0.21

4.2. Enzyme Inhibition Activity

WMLE showed strong, dose-dependent inhibition of both enzymes, with greater potency against α-glucosidase

Table 2: Inhibitory Activity (IC_{50}) of WMLE and Acarbose

Sample	α-Glucosidase IC_{50} (µg/mL)	α-Amylase IC_{50} (µg/mL)	Selectivity Ratio
WMLE	38.5 ± 2.1	245.7 ± 15.3	6.4
Acarbose	12.8 ± 0.9*	185.4 ± 12.6*	14.5

* $p < 0.05$ vs. WMLE.

4.3. Time-Kinetics of Inhibition

Maximum α-glucosidase inhibition by WMLE was achieved after 15–20 minutes of pre-incubation, indicating time-dependent binding.

4.4. Glucose Uptake in L6 Cells

WMLE stimulated basal glucose uptake up to 2.1-fold at 100 µg/mL. Co-treatment with insulin showed synergistic enhancement (3.0-fold increase, $p < 0.001$).

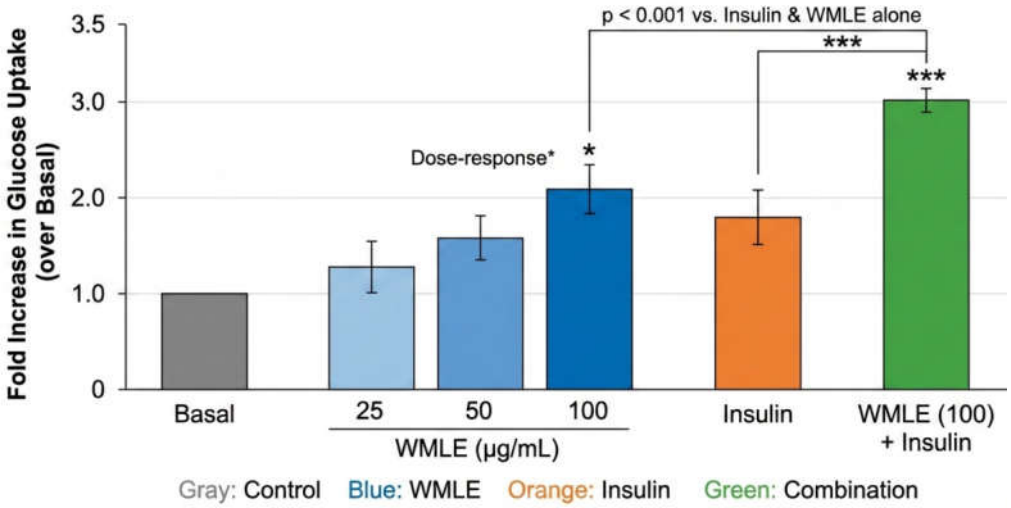

Figure 1: Glucose Uptake Stimulation by WMLE in L6 Myotubes (Bar chart showing dose-response and synergy with insulin)

4.5. Synergy Analysis

The reconstituted mixture of AQF and EAF fractions showed greater inhibition (IC_{50} = 42.5 µg/mL) than predicted, with a CI of 0.78, indicating synergism.

Table 3: Synergy Analysis of WMLE Fractions

Sample	Description	IC_{50} (µg/mL)	CI at IC_{50}
AQF	Aqueous Fraction	85.4 ± 6.7	–
EAF	Ethyl Acetate Fraction	112.3 ± 9.8	–
Recon	AQF + EAF	42.5 ± 3.2	0.78

5. Discussion

This study confirms that WMLE is a potent inhibitor of carbohydrate-digesting enzymes, primarily due to its high DNJ content. Its selectivity for α-glucosidase over α-amylase is clinically favorable, potentially reducing gastrointestinal side effects[11]. The time-dependent inhibition suggests optimal pre-meal administration ~15–20 minutes before food intake.

The significant stimulation of glucose uptake in L6 cells, especially the synergy with insulin, highlights WMLE's role in improving insulin sensitivity—a critical mechanism in T2DM

management[12]. The synergy between DNJ-rich and flavonoid-rich fractions supports the holistic use of the whole extract, as multi-component interactions enhance overall efficacy[13].

These findings align with traditional use and previous studies but extend the evidence by providing direct comparison with acarbose, temporal kinetics, and mechanistic insights into peripheral glucose disposal.

6. Conclusion

Standardized White Mulberry Leaf Extract demonstrates dual antidiabetic mechanisms: inhibition of intestinal α-glucosidase to reduce postprandial glucose spikes, and enhancement of insulin-mediated glucose uptake in skeletal muscle. The synergistic interactions among its phytoconstituents underscore the advantage of using the whole extract over isolated compounds. WMLE represents a promising, multi-target botanical adjunct for managing T2DM, particularly for postprandial hyperglycemia and insulin resistance. Further in vivo studies are warranted to validate these findings.

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Conflict of Interest Statement

The author declares no conflict of interest.

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