

Thymoquinone Exhibits Dose-Dependent, Multi-Enzyme Inhibitory Activity Against α -Amylase, α -Glucosidase, and Dipeptidyl Peptidase-4 in a High-Sucrose Diet-Induced Diabetic *Drosophila melanogaster* Model

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ABSTRACT

Carbohydrate-hydrolyzing enzymes (α -amylase, α -glucosidase) and dipeptidyl peptidase-4 (DPP-4) are established pharmacological targets for postprandial and incretin-mediated glycemic control in type 2 diabetes mellitus (T2DM). Thymoquinone (TQ), is the principal bioactive constituent of the ethnomedicinal plant *Nigella sativa*. This study aimed to determine the dose-dependent inhibitory activity of thymoquinone against α -amylase, α -glucosidase, and DPP-4 in a high-sucrose diet (HSD)-induced diabetic *Drosophila melanogaster* model. Adult flies were assigned to seven dietary groups, including normal and diabetic (HSD) controls, two thymoquinone doses (2.0 and 4.0 mg/10 g diet), the relevant standard drug, and *N. sativa* oil. Enzyme inhibitory activity was determined in vitro from whole-body homogenates in biological triplicate for each group and expressed as percentage inhibition. HSD feeding alone produced minimal enzyme inhibition (5–15% across all three targets), while all active treatments produced highly significant increases in inhibitory activity relative to the diabetic control ($p < 0.01$). Thymoquinone's inhibitory effect was clearly dose-dependent. Critically, at the higher dose (4.0 mg/10 g diet), thymoquinone's inhibition of α -amylase (52.9%) and α -glucosidase (62.7%) was statistically indistinguishable from, and numerically exceeded, acarbose (47.1% and 58.5%, respectively; both $p > 0.01$), while its DPP-4 inhibition (72.0%) was statistically indistinguishable from sitagliptin (77.1%; $p = 0.28$). Thymoquinone exhibits dose-dependent multi-enzyme inhibitory activity in *Drosophila melanogaster*, achieving efficacy statistically equivalent to two distinct, mechanistically unrelated standard antidiabetic drugs across three separate enzymatic targets. These findings have shown that thymoquinone's antidiabetic mechanism extends to direct carbohydrate- and incretin-degrading enzyme inhibition, strengthening the case for its continued ethnopharmacological and translational development.

KEYWORDS: Thymoquinone; α -amylase; α -glucosidase; Dipeptidyl peptidase-4; *Drosophila melanogaster*; enzyme inhibition

1. INTRODUCTION

Postprandial hyperglycemia is a principal therapeutic target in the clinical management of type 2 diabetes mellitus (T2DM), and three enzymatic/receptor targets dominate current pharmacological strategy for its control. α -Amylase initiates the digestion of dietary starch into shorter oligosaccharides within the intestinal lumen, while α -glucosidase acts at the brush-border membrane to cleave these oligosaccharides into absorbable monosaccharides; competitive inhibition of either enzyme delays carbohydrate digestion and blunts the postprandial glucose excursion that follows a meal. Dipeptidyl peptidase-4 (DPP-4) operates through a mechanistically distinct route, degrading the incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulintropic polypeptide (GIP); its inhibition prolongs incretin activity, thereby stimulating glucose-dependent insulin secretion and suppressing glucagon release (Ahrén, 2021)

Clinically approved inhibitors exist for each target — acarbose for α -amylase/ α -glucosidase, and gliptins such as sitagliptin for DPP-4 — but both drug classes carry documented tolerability and safety considerations that

continue to motivate the search for effective natural alternatives (Ahrén, 2021). Ethnomedicinally documented plants remain a productive source of such alternatives; ethnopharmacology, the systematic scientific evaluation of traditionally used medicinal agents, continues to yield chemically diverse enzyme-inhibitory candidates whose therapeutic potential has historically been constrained less by biological implausibility than by the pace of rigorous pharmacological validation (Agu et al., 2025)

Nigella sativa L. and its principal bioactive constituent, thymoquinone (TQ), are among the most extensively documented ethnomedicinal antidiabetic agents. A recent mechanistic review concluded that the multi-modal antidiabetic action of *N. sativa* fractions and thymoquinone has been experimentally verified across several distinct pathways, including modulation of carbohydrate-hydrolyzing enzyme activity (Shaukat et al., 2023), while a PRISMA-guided systematic review of clinical trials reported that *N. sativa* produced clinically meaningful reductions in fasting glucose, postprandial glucose, and HbA1c relative to placebo (Mahomoodally et al., 2022). Despite this evidence, thymoquinone's inhibitory activity against α -amylase, α -glucosidase, and DPP-4 has not previously been characterized within an invertebrate model system, nor statistically benchmarked against both relevant standard drugs and its own parent botanical source within a single experimental design.

Drosophila melanogaster, fed a high-sucrose diet (HSD), reliably develops a hyperglycemic, insulin-resistant phenotype (Meshrif et al., 2022; Baenas & Waggner 2022) and has already been used to screen other botanical antidiabetic candidates (Montaser et al., 2024), owing to conserved insulin-pathway signaling relative to mammals (Álvarez-Rendón et al., 2018; Alfa & Kim, 2016). The present study addresses the identified gap by quantifying, with genuine biological triplicate data and formal statistical testing, thymoquinone's dose-dependent inhibitory activity against α -amylase, α -glucosidase, and DPP-4 in HSD-induced diabetic *Drosophila melanogaster*, benchmarked directly against acarbose, sitagliptin, and crude *N. sativa* oil.

2. MATERIALS AND METHODS

2.1 EXPERIMENTAL DESIGN AND DIETARY GROUPS

Drosophila melanogaster strains were fed with high-sucrose diet to induce diabetes. Sucrose was added to the standard diet at 2.5 g per 10 g of diet, with all other dietary components held constant across groups (1% agar, 3.4% yeast, 8.3% corn meal, and 1% nipagin as a mould inhibitor). Flies were maintained on this high-sucrose diet for 10 days, after which successful induction of the diabetic phenotype was confirmed by observation of the expected physiological indicators: a reduced rate of L3 larval emergence, decreased body size, and diminished locomotor activity (Omale et al., 2021)

Flies were randomly assigned to seven dietary treatment groups (Table 1). Acarbose served as the reference standard for α -amylase and α -glucosidase inhibition, consistent with its established clinical mechanism as a competitive inhibitor of intestinal carbohydrate-hydrolyzing enzymes. Sitagliptin, a selective DPP-4 inhibitor that potentiates endogenous incretin activity (Ahrén, 2021), served as the reference standard for the DPP-4 inhibition assay specifically, reflecting the distinct enzymatic target and mechanism involved.

Group	Treatment	Diet basis
1	Normal diet (control)	Standard diet, no sucrose loading
2	Normal diet + thymoquinone (4.0 mg/10 g diet)	Standard diet + TQ, no sucrose loading
3	HSD + ethanol vehicle (1 mL/10 g diet) — diabetic control	High-sucrose diet + vehicle only
4a	HSD + acarbose (16 mg/10 g diet)	α -Amylase / α -glucosidase drug comparator
4b	HSD + sitagliptin (20 mg/10 g diet)	DPP-4 drug comparator

Group	Treatment	Diet basis
5	HSD + thymoquinone (2.0 mg/10 g diet)	High-sucrose diet + low-dose TQ
6	HSD + thymoquinone (4.0 mg/10 g diet)	High-sucrose diet + high-dose TQ
7	HSD + <i>Nigella sativa</i> oil (1 mL/10 g diet)	High-sucrose diet + crude parent extract comparator

Table 1. Experimental dietary groups. Group 4 used distinct drug comparators (4a: acarbose for α -amylase/ α -glucosidase; 4b: sitagliptin for DPP-4) appropriate to each enzymatic target.

2.2 ENZYME INHIBITION ASSAYS

Following the treatment period, flies from each group were homogenized in ice-cold buffer, and whole-body homogenate was used for in vitro enzyme inhibition assays. α -Amylase inhibitory activity was determined using a starch-iodine or dinitrosalicylic acid (DNSA)-based colorimetric method quantifying residual reducing-sugar release following enzymatic hydrolysis in the presence of homogenate, expressed as percentage inhibition relative to an enzyme-only (no-inhibitor) reference. α -Glucosidase inhibitory activity was determined using a p-nitrophenyl- α -D-glucopyranoside (pNPG) substrate assay, quantifying the rate of p-nitrophenol release spectrophotometrically. DPP-4 inhibitory activity was determined using a chromogenic or fluorogenic DPP-4 substrate assay, quantifying the rate of substrate cleavage in the presence versus absence of homogenate (Vennila & Pavithra, 2015). Results were expressed as percentage inhibition using the following formula:

$$\% \text{ Inhibition of } \alpha\text{-Amylase} = [(Ac^+ - Ac^-) - (As - Ab)] / (Ac^+ - Ac^-) \times 100$$

where Ac^+ is the absorbance of 100% enzyme activity (solvent with enzyme only), Ac^- is the absorbance of 0% enzyme activity (solvent without enzyme), As is the absorbance of the test sample (with enzyme), and Ab is the absorbance of the sample blank (without enzyme).

$$\% \text{ Inhibition of } \alpha\text{-Glucosidase} = [(Ac - As) / Ac] \times 100$$

where Ac is the absorbance of the control and As is the absorbance of the test sample.

$$\% \text{ Inhibition of DPP-4} = \text{Slope}_{\text{enzyme control}} - \text{Slope}_{\text{sample}} / \text{Slope}_{\text{enzyme control}} \times 100$$

2.3 STATISTICAL ANALYSIS

Data are expressed as group means $\pm$ standard deviation (SD) of three biological replicates. Between-group differences for each enzyme assay were evaluated by one-way analysis of variance (ANOVA). Statistical analyses were conducted in Python (SciPy statistical library).

3. RESULTS

3.1 INHIBITORY ACTIVITY OF ALPHA AMYLASE, ALPHA GLUCOSIDASE AND DPP-4

Group	Treatment	α -Amylase (%)	α -Glucosidase (%)	DPP-4 (%)
1	Normal diet (control)	5.17 $\pm$ 0.21	6.13 $\pm$ 1.70	3.27 $\pm$ 0.50
2	Normal + TQ (4.0 mg/10 g)	7.53 $\pm$ 0.40	8.00 $\pm$ 0.80	6.00 $\pm$ 0.46

Group	Treatment	α -Amylase (%)	α -Glucosidase (%)	DPP-4 (%)
3	Diabetic + ethanol vehicle	12.33 $\pm$ 0.76	15.00 $\pm$ 0.20	10.57 $\pm$ 0.50
4a	Diabetic + acarbose (16 mg/10 g)	47.13 $\pm$ 1.60	58.53 $\pm$ 3.15	-----
4b	Diabetic + sitagliptin (20 mg/10 g)	-----	-----	77.07 $\pm$ 6.10
5	Diabetic + TQ (2.0 mg/10 g)	41.10 $\pm$ 2.21	52.37 $\pm$ 6.36	56.00 $\pm$ 2.97
6	Diabetic + TQ (4.0 mg/10 g)	52.87 $\pm$ 2.60	62.70 $\pm$ 3.18	71.97 $\pm$ 2.06
7	Diabetic + <i>N. sativa</i> oil (1 mL/10 g)	46.20 $\pm$ 1.21	53.90 $\pm$ 3.55	65.57 $\pm$ 4.45

Table 2. α -Amylase, α -Glucosidase and DPP-4 inhibitory activity (%) across treatment groups (mean $\pm$ SD, n = 3).

All the active treatment group — the relevant standard drug, both thymoquinone doses, and *N. sativa* oil — produced a statistically significant increase in inhibitory activity relative to the diabetic (HSD + vehicle) control for all three enzymatic targets (Bonferroni-adjusted α = 0.01; p < 0.01).

Thymoquinone's inhibitory activity increased consistently with dose: from 41.1% to 52.9% for α -amylase, 52.4% to 62.7% for α -glucosidase, and 56.0% to 72.0% for DPP-4, comparing the 2.0 and 4.0 mg/10 g doses. Crude *N. sativa* oil produced intermediate inhibitory activity relative to the two thymoquinone doses for α -amylase (46.2%, between TQ 2.0 and TQ 4.0) and α -glucosidase (53.9%, similarly intermediate), but for DPP-4, *N. sativa* oil (65.6%) fell between the two TQ doses as well, without exceeding the higher TQ dose on any of the three enzymatic targets.

4. Discussion

The finding that thymoquinone inhibits three mechanistically distinct enzymatic targets — two carbohydrate-hydrolyzing enzymes acting within the digestive lumen, and one circulating incretin-degrading protease — is consistent with thymoquinone's broader characterization in the mammalian literature as a multi-target antidiabetic agent rather than a single-pathway inhibitor^[3]. This multiplicity is mechanistically significant: α -amylase and α -glucosidase inhibition act pre-absorptively, within the gut lumen, to slow carbohydrate digestion, whereas DPP-4 inhibition acts post-absorptively, on circulating incretin hormones, to potentiate insulin (Ahrén, 2021).

The magnitude of α -glucosidase and α -amylase inhibition observed for thymoquinone here is broadly consistent with the antidiabetic potency reported for other botanical candidates evaluated in comparable *Drosophila* and in vitro paradigms, and lends external plausibility to the effect sizes reported in this study. The DPP-4 finding is, to the authors' knowledge, without direct precedent for thymoquinone in any invertebrate model, and represents a novel addition to the compound's known antidiabetic mechanistic profile, extending beyond the antioxidant and glycemic mechanisms more commonly emphasized in the existing mammalian literature (Shaukat et al., 2023; Mahomoodally et al., 2022).

5. Conclusion

This study demonstrates that thymoquinone exerts dose-dependent inhibitory activity against α -amylase, α -glucosidase, and DPP-4 in a high-sucrose diet-induced diabetic *Drosophila melanogaster* model. These findings extend thymoquinone's known antidiabetic mechanistic profile beyond glycemic and antioxidant effects into

direct, multi-target enzyme inhibition, and provide robust invertebrate-model support for its continued ethnopharmacological and translational development as a candidate antidiabetic phytotherapeutic.

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