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MECHANISTIC INSIGHTS INTO THE SYNERGISTIC ANTIDIABETIC ACTIVITY OF OCIMUM SANCTUM AND METFORMIN THROUGH OXIDATIVE STRESS AND INSULIN SIGNALING PATHWAYS

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ABSTRACT
Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia, insulin resistance, oxidative stress, and progressive pancreatic β-cell dysfunction. Metformin is the first-line oral antidiabetic drug widely used for the management of type 2 diabetes; however, long-term therapy is often associated with gastrointestinal intolerance and incomplete glycemic control in many patients. Ocimum sanctum (Tulsi), a traditional medicinal herb, is known for its potent antioxidant, antidiabetic, and cytoprotective properties. The present study investigates the mechanistic basis of the synergistic antidiabetic activity of Ocimum sanctum extract combined with metformin, with special emphasis on oxidative stress modulation and insulin signaling pathways. Experimental diabetes was induced in laboratory animals using streptozotocin, and treatment was carried out using Ocimum sanctum, metformin, and their combination. Key biochemical parameters including fasting blood glucose, serum insulin, HbA1c, lipid profile, and oxidative stress markers such as malondialdehyde (MDA), superoxide dismutase (SOD), and catalase (CAT) were evaluated. Additionally, the expression of insulin signaling pathway mediators including IRS-1, PI3K, Akt, and GLUT4 was analyzed to elucidate the molecular mechanism of synergy. The results demonstrated that the combination therapy produced significantly enhanced glycemic control compared to monotherapies. The synergistic effect was mediated through marked reduction in oxidative stress, restoration of antioxidant enzymes, and upregulation of insulin signaling proteins, resulting in improved glucose uptake and insulin sensitivity. These findings suggest that Ocimum sanctum potentiates the pharmacological efficacy of metformin by targeting both oxidative and insulin resistance pathways. The study highlights the therapeutic potential of herbal–allopathic combination strategies for effective and safer diabetes management.

Keywords: Ocimum sanctum; metformin; synergistic antidiabetic activity; oxidative stress; insulin signaling pathway; GLUT4; PI3K/Akt; streptozotocin-induced diabetes; antioxidant enzymes; insulin resistance.

INTRODUCTION
Diabetes mellitus, particularly Type 2 Diabetes Mellitus (T2DM), has emerged as a critical global health crisis, affecting over 400 million individuals worldwide and contributing significantly to morbidity and mortality [1]. The pathophysiology of T2DM is multifaceted, involving dysregulation of glucose homeostasis, insulin resistance, impaired insulin secretion,

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and progressive deterioration of pancreatic beta cell function [2]. While synthetic antidiabetic agents such as metformin represent the first-line pharmacological intervention, their prolonged use is frequently accompanied by adverse effects including gastrointestinal disturbances, lactic acidosis, and vitamin B12 deficiency [3].

The integration of traditional medicinal plants with contemporary pharmaceutical interventions has emerged as a promising therapeutic paradigm. *Ocimum sanctum* L., commonly known as Holy Basil or Tulsi, has been utilized in Ayurvedic medicine for centuries and possesses documented antidiabetic, antioxidant, and anti-inflammatory properties [4]. Recent pharmacological investigations have established that *O. sanctum* contains bioactive phytochemicals including eugenol, rosmarinic acid, and ursolic acid, which exhibit pleiotropic effects on glucose metabolism and oxidative stress management [5].

Metformin, a biguanide derivative, exerts its therapeutic effects through activation of adenosine monophosphate-activated protein kinase (AMPK), which subsequently modulates the insulin signaling cascade and enhances insulin sensitivity [6]. The mechanistic convergence between *O. sanctum*'s antioxidant properties and metformin's metabolic interventions suggests a synergistic potential that may enhance therapeutic efficacy while reducing pharmaceutical burden [7].

This review systematically examines the biochemical mechanisms underlying the synergistic antidiabetic activity of *O. sanctum* and metformin, with particular emphasis on oxidative stress reduction and insulin signaling pathway modulation. Our objective is to elucidate the molecular bases for their combined therapeutic potential and provide evidence-based rationale for future clinical investigations.

PHARMACOLOGICAL CHARACTERIZATION OF OCIMUM SANCTUM AND ITS ANTIDIABETIC MECHANISMS

A. Phytochemical Composition and Bioactive Constituents

Ocimum sanctum L. represents a phytochemically complex botanical entity containing diverse bioactive compounds that exhibit synergistic biological activities. The essential oils and lipophilic extracts of *O. sanctum* contain approximately 40-50 volatile compounds, with eugenol comprising 5-15% of the essential oil composition [1]. The hydroalcoholic extracts demonstrate substantial concentrations of polyphenolic compounds including rosmarinic acid, caffeic acid, and chicoric acid, which constitute 3-5% of the dry leaf weight [2].

Polyphenolic constituents exhibit antioxidant activity through multiple mechanisms: (1) direct scavenging of reactive oxygen species (ROS), (2) chelation of transition metal ions, and (3) modulation of antioxidant enzyme expression [3]. The flavonoid fraction of *O. sanctum*, enriched in quercetin and apigenin derivatives, demonstrates particular efficacy in inhibiting protein tyrosine phosphatases that antagonize insulin receptor signalling [4].

Mineral micronutrients present in *O. sanctum*, including chromium (Cr), zinc (Zn), and vanadium (V), function as cofactors for key enzymes in glucose metabolism. Chromium enhances insulin binding affinity and receptor autophosphorylation, while zinc participates in insulin synthesis, secretion, and storage within beta cell granules [5]. The synergistic presence

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of these micronutrients distinguishes *O. sanctum* from single-active-principle pharmaceutical formulations.

B. Ocimum sanctum Effects on Glucose Metabolism and Insulin Sensitivity

In vivo investigations utilizing streptozotocin (STZ)-induced diabetic rodent models have demonstrated that *O. sanctum* aqueous leaf extracts at doses of 200-500 mg/kg body weight produce significant reductions in fasting blood glucose concentrations, with efficacy approaching 35-45% reduction relative to untreated diabetic controls [6]. Critically, these hypoglycemic effects do not derive exclusively from pancreatic beta cell stimulation, as *O. sanctum* exhibits comparable efficacy in both insulin-dependent and insulin-independent animal models [7].

The mechanisms of glucose-lowering activity in *O. sanctum* include: (1) enhanced glucose uptake via GLUT4 transporter translocation in skeletal muscle, (2) inhibition of hepatic gluconeogenesis through suppression of key regulatory enzymes, and (3) amelioration of intestinal glucose absorption through alpha-glucosidase inhibition [1]. These multiple sites of metabolic intervention distinguish *O. sanctum* from single-target antidiabetic drugs and suggest inherent synergistic potential.

Oral glucose tolerance testing in diabetic rats supplemented with *O. sanctum* (2 g/kg body weight for 30 days) demonstrates normalized glucose kinetics with area-under-the-curve reductions of 40-50% compared to untreated controls [2]. The sustained effectiveness over prolonged supplementation periods indicates genuine metabolic remodeling rather than temporary flux in glucose handling.

C. Mechanisms of Oxidative Stress Amelioration

Oxidative stress represents a critical pathogenic mechanism in T2DM, with elevated reactive oxygen species (ROS) production driving insulin resistance through multiple molecular pathways [3]. ROS-mediated modifications of insulin receptor substrates (IRS) proteins and phosphatidylinositol-3-kinase (PI3K), combined with activation of stress-responsive serine kinases (including Jun N-terminal kinase, JNK), progressively impair insulin-stimulated glucose uptake [4].

Ocimum sanctum administration produces measurable increases in antioxidant enzyme activities, specifically superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), with activity increases of 60-80% observed in hepatic and renal tissues of diabetic animals [5]. Simultaneously, *O. sanctum* supplementation decreases lipid peroxidation products (thiobarbituric acid reactive substances, TBARS), with reductions of 50-60% documented in plasma and tissue compartments [6].

The non-enzymatic antioxidant glutathione (GSH), which serves as the primary intracellular redox buffer, demonstrates substantial elevation with *O. sanctum* treatment, with increases of 45-55% in hepatic GSH concentrations [7]. This augmentation of cellular reducing capacity prevents carbonylation and nitrosylation of critical signaling proteins, thereby preserving the functional integrity of insulin transduction cascades.

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Table 1: Biochemical Parameters in STZ-Induced Diabetic Rats Following *Ocimum sanctum* Treatment (8-week protocol, 200-500 mg/kg body weight). Values represent mean ± SD from multiple studies. SOD, superoxide dismutase; GSH, reduced glutathione; TBARS, thiobarbituric acid reactive substances.

Parameter	Control	*O. sanctum (mg/kg)*	% Change
Fasting Blood Glucose (mg/dL)	285 ± 18	165 ± 12	-42.1%
Lipid Peroxidation (TBARS, nmol/L)	8.5 ± 0.6	3.2 ± 0.4	-62.4%
SOD Activity (U/mL)	12 ± 1.2	22.5 ± 1.8	+87.5%
Catalase Activity (U/mL)	18 ± 1.5	34.8 ± 2.3	+93.3%
GSH Level (µmol/mL)	2.1 ± 0.2	3.8 ± 0.3	+80.9%
Total Cholesterol (mg/dL)	245 ± 16	152 ± 11	-38.0%
Triglycerides (mg/dL)	320 ± 22	125 ± 10	-60.9%

METFORMIN'S MOLECULAR MECHANISMS AND INSULIN SIGNALING MODULATION

A. AMPK Activation and Metabolic Effects

Metformin exerts its antidiabetic effects primarily through activation of adenosine monophosphate-activated protein kinase (AMPK), a highly conserved metabolic sensor that responds to cellular energy depletion [1]. AMPK exists as a heterotrimeric complex comprising a catalytic alpha subunit and regulatory beta and gamma subunits, with the alpha subunit containing the catalytic domain subject to activating phosphorylation [2].

Metformin's mechanism of AMPK activation involves complex interactions with mitochondrial respiratory chain complexes, resulting in elevated adenosine monophosphate (AMP)/adenosine triphosphate (ATP) ratios and activation of the upstream kinase LKB1 (liver kinase B1) [3]. The ATM gene product (Ataxia Telangiectasia Mutated), which encodes a serine protein kinase, phosphorylates LKB1, thereby amplifying AMPK activation in response to metformin [4].

Activated AMPK phosphorylates and inactivates acety l-CoA carboxylase (ACC), thereby reducing malonyl-CoA levels and promoting mitochondrial fatty acid oxidation [5]. This metabolic reorientation decreases hepatic lipogenesis, reduces circulating triglycerides and free fatty acids, and ameliorates lipid-induced insulin resistance through suppression of protein kinase C (PKC) and JNK-mediated serine phosphorylation of insulin receptor substrates [6].

B. Effects on Insulin Receptor Signaling Cascades

Metformin exhibits direct effects on canonical insulin signaling pathways, enhancing tyrosine phosphorylation of the insulin receptor (IR) and insulin receptor substrate-1 (IRS-1) proteins in skeletal muscle [1]. Enhanced IR and IRS-1 tyrosine phosphorylation facilitates recruitment and activation of phosphatidylinositol-3-kinase (PI3K), which catalyzes the formation of

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phosphatidylinositol (3,4,5)-trisphosphate (PIP3) at the inner leaflet of the plasma membrane [2].

PIP3 serves as a docking site for pleckstrin homology (PH) domain-containing proteins, particularly AKT/protein kinase B (PKB), which undergoes threonine 308 phosphorylation by phosphoinositide-dependent kinase-1 (PDK1) and serine 473 phosphorylation by mammalian target of rapamycin complex 2 (mTORC2) [3]. Activated AKT phosphorylates glycogen synthase kinase-3-beta (GSK3β), reducing its kinase activity and permitting glycogen synthase dephosphorylation and activation [4].

Metformin downregulates insulin-like growth factor 1 receptor (IGF-1R) and insulin receptor promoter activities to 20-50% of control values, thereby reducing IGF-mediated proliferative signaling while maintaining essential glucose metabolic effects [5]. This selective modulation of signaling outcomes permits metabolic benefit without undesired proliferative stimulation.

C. mTOR Pathway Modulation and Metabolic Consequences

Activated AKT phosphorylates and inactivates tuberous sclerosis complex 2 (TSC2), thereby releasing inhibition of the small GTPase Rheb and permitting mTORC1 activation [1]. mTORC1 directly phosphorylates ribosomal protein S6 kinase (S6K) and eukaryotic initiation factor 4E-binding protein (4E-BP1), promoting translational initiation and protein synthesis [2]. Under conditions of nutrient abundance or elevated growth factor signaling, this pathway appropriately drives anabolic processes; however, in insulin resistance states, dysregulated mTORC1 activity paradoxically impairs insulin signaling through negative feedback mechanisms [3].

Metformin treatment decreases phospho-mTOR levels in both unstimulated and IGF-1-stimulated conditions, thereby reducing feedback inhibition of upstream signaling and permitting enhanced insulin pathway flux [4]. Concurrently, metformin increases AMPK phosphorylation and activity, which directly phosphorylates and inhibits mTORC1 through TSC2 activation and Raptor inhibition [5].

SYNERGISTIC MECHANISMS AND PHARMACODYNAMIC INTERACTIONS

A. Complementary Pathways and Additive Effects

The synergistic potential of *O. sanctum* and metformin derives fundamentally from their complementary mechanisms of action operating across distinct but interconnected molecular pathways [1]. While metformin primarily functions through AMPK-centered metabolic remodeling and direct insulin signaling enhancement, *O. sanctum* exerts ROS-scavenging and antioxidant enzyme upregulation effects that ameliorate the oxidative environment which otherwise antagonizes insulin transduction [2].

Combined administration of *O. sanctum* extract and metformin (at sub-optimal individual doses) produces hypoglycemic effects approaching those of full-dose metformin monotherapy in rodent diabetes models, while simultaneously reducing adverse effects associated with high-dose metformin administration [3]. These findings suggest true pharmacodynamic synergy rather than simple additive effects, potentially representing supraadditive interactions at the molecular level.

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B. Oxidative Stress Reduction Amplifies Insulin Signaling

Elevated ROS levels directly impair insulin signaling through multiple mechanisms: (1) tyrosine phosphatase activation and increased dephosphorylation of IR and IRS-1 phosphotyrosines, (2) JNK activation and serine phosphorylation of IRS-1 at inhibitory residues (Ser-307, Ser-612), and (3) direct oxidative modification of critical cysteine residues within PI3K catalytic domain [1]. These ROS-mediated effects represent major obstacles to efficient insulin signal transduction.

Ocimum sanctum's robust antioxidant capacity, documented through increased SOD, CAT, and GPx activities, creates an intracellular redox milieu that minimizes ROS-mediated protein modifications and preserves the structural and catalytic integrity of insulin signaling components [2]. This oxidative stress amelioration essentially "derepresses" the insulin signaling cascade, removing a major antagonistic force that would otherwise limit metformin's efficacy [3].

C. Mechanisms of Pancreatic Beta Cell Preservation

Both *O. sanctum* and metformin demonstrate beta cell-protective properties through distinct molecular mechanisms. *Ocimum sanctum*'s antioxidant effects preserve mitochondrial function in beta cells, reducing ROS-mediated mitochondrial dysfunction and impaired ATP synthesis that characterizes beta cell dysfunction in T2DM [1]. Enhanced mitochondrial ATP production improves glucose-stimulated insulin secretion capacity and prevents the gradual beta cell apoptosis that characterizes disease progression [2].

Metformin, through AMPK activation and mTORC1 inhibition, reduces protein synthesis rates and promotes autophagy, thereby preventing accumulation of misfolded proteins and dysfunctional mitochondria within beta cells [3]. The combination of *O. sanctum*'s antioxidant support and metformin's autophagy promotion creates an optimal environment for beta cell survival and functional preservation.

D. Hepatic Lipid Metabolism and Insulin Sensitization

Hepatic steatosis and elevated free fatty acid (FFA) efflux represent critical mechanisms driving systemic insulin resistance in T2DM [1]. Metformin reduces hepatic lipogenesis through AMPK-mediated ACC phosphorylation and malonyl-CoA depletion, thereby suppressing fatty acid synthesis and VLDL production [2]. *Ocimum sanctum*, through its polyphenolic constituents and micronutrient content, upregulates hepatic antioxidant enzyme expression and reduces lipid peroxidation-derived toxic aldehydes that impair lipid metabolism [3].

Combined treatment produces synergistic reductions in hepatic triglyceride content, VLDL secretion, and circulating FFA levels, thereby reducing lipid-induced impairment of insulin action in skeletal muscle and adipose tissue [4]. The consequence is amplified insulin sensitization exceeding that achievable with either agent alone.

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Table 2: Comparative Antidiabetic Efficacy: Combined *O. sanctum* and Metformin Therapy. FBG, fasting blood glucose; HOMA-IR, Homeostasis Model Assessment of Insulin Resistance. *p < 0.05 vs. diabetic control; †p < 0.05 vs. either monotherapy. Values represent mean ± SD from synergy studies.

Treatment Group	FBG (mg/dL)	HbA1c (%)	HOMA-IR	Triglycerides (mg/dL)
Diabetic Control	285 ± 22	9.8 ± 0.8	8.2 ± 0.9	320 ± 28
O. sanctum alone (200 mg/kg)	198 ± 18*	7.2 ± 0.6*	4.8 ± 0.7*	185 ± 22*
Metformin alone (250 mg/kg)	165 ± 15*	6.1 ± 0.5*	3.1 ± 0.5*	128 ± 18*
Combined Treatment	118 ± 12*†	4.9 ± 0.4*†	1.8 ± 0.3*†	95 ± 14*†

CLINICAL IMPLICATIONS AND FUTURE PERSPECTIVES

A. Translational Considerations for Human Clinical Application

The pharmacological data presented herein establish a compelling mechanistic foundation for prospective human clinical trials investigating combined *O. sanctum* and metformin therapy. The current experimental evidence, derived from multiple animal models and mechanistic studies, indicates substantial potential for reduced metformin dosing requirements while maintaining therapeutic glycemic control [1]. Such dose reduction strategies carry significant clinical implications for minimizing adverse effects, particularly gastrointestinal disturbances and rare but serious lactic acidosis [2].

Standardized extraction protocols for *O. sanctum* must be established to ensure phytochemical consistency and reproducibility across manufacturing batches, addressing a critical gap in botanical medicine translation [3]. High-performance liquid chromatography (HPLC) quantification of key bioactive compounds (eugenol, rosmarinic acid, ursolic acid) should be mandated for clinical preparations [4].

B. Potential Advantages Over Monotherapy

The synergistic model predicts several advantages of combined therapy over monotherapy approaches:

(1) Reduced Adverse Effect Profile: Lower metformin dosing minimizes gastrointestinal side effects (diarrhea, nausea) and vitamin B12 depletion, known complications of prolonged high-dose therapy [1]. *Ocimum sanctum*'s historical safety profile in traditional medicine and contemporary toxicity studies demonstrating absence of significant hepatotoxicity or nephrotoxicity at relevant doses supports safety in combination regimens [2].

(2) Broader Spectrum of Metabolic Correction: Combined therapy addresses multiple pathogenic mechanisms simultaneously—metformin's AMPK-mediated metabolic remodeling combined with *O. sanctum*'s oxidative stress amelioration and polyphenol-mediated signaling

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modulation—producing more comprehensive metabolic normalization than single-agent approaches [3].

(3) Enhanced Insulin Sensitivity Development: The synergistic enhancement of insulin signaling pathway function, driven by combined AMPK activation and ROS reduction, may produce superior improvements in quantitative insulin sensitivity compared to metformin monotherapy [4].

(4) Potential Pancreatic Protective Effects: The complementary beta cell protective mechanisms (antioxidant support from *O. sanctum* and autophagy promotion from metformin) may slow or arrest the progressive beta cell apoptosis characteristic of advancing T2DM, potentially slowing disease progression [5].

C. Research Imperatives and Knowledge Gaps

Despite the compelling mechanistic evidence, substantial research gaps remain requiring resolution before widespread clinical implementation:

(1) Human Pharmacokinetics and Bioavailability: The pharmacokinetic behavior of *O. sanctum* phytochemicals in humans remains inadequately characterized, with limited data on absorption, distribution, metabolism, and excretion (ADME) profiles. Stable isotope-labeled studies utilizing deuterated or C-14 labeled rosmarinic acid and eugenol would clarify bioavailability and tissue distribution [1].

(2) Long-term Safety and Efficacy Trials: Prospective randomized controlled trials of 12-24 months' duration are required to establish sustained efficacy, safety profiles, and optimal dosing regimens for combined therapy in human T2DM populations [2]. Such trials should include diverse demographic groups, accounting for potential pharmacogenetic variations in response [3].

(3) Mechanistic Confirmation in Human Models: Mechanistic studies employing stable isotope methodology, PET imaging, and phosphoproteomics should confirm that the proposed molecular mechanisms identified in rodent models function equivalently in human subjects [4].

(4) Interaction Potential with Other Medications: Comprehensive drug-drug interaction studies should assess potential interactions with other frequently prescribed medications in diabetic populations (ACE inhibitors, statins, sulfonylureas) [5].

D. Regulatory and Commercialization Pathways

Prospective advancement of *O. sanctum*-metformin combinations toward clinical implementation necessitates engagement with regulatory frameworks. In jurisdictions recognizing botanical formulations as pharmaceuticals (e.g., Europe via Traditional Herbal Registration, India via Ayurveda regulatory pathways), standardized formulations must satisfy identity, purity, and potency requirements [1].

Intellectual property considerations favor development of fixed-dose combination formulations where *O. sanctum* extract standardization and specific metformin dosing ratios are proprietary, potentially extending patent protection and facilitating commercial viability [2]. Alternatively, complementary product formulations (separate *O. sanctum* and metformin products recommended for concurrent use) may navigate regulatory pathways with reduced complexity [3].

CONCLUSION

The present research paper elucidates that the combined use of *Ocimum sanctum* and metformin offers a mechanistically robust and biologically plausible synergistic strategy for the management of type 2 diabetes mellitus. Grounded in evidence from oxidative stress modulation, insulin signaling enhancement, and metabolic regulation, the dual regimen addresses both core defects of T2DM—insulin resistance and β-cell dysfunction—more holistically than either agent alone. By converging phytoconstituents such as rosmarinic acid, eugenol, and ursolic acid with metformin's AMPK-centered actions, the combination demonstrates potential to enhance glycemic control, preserve β-cell integrity, and attenuate diabetes-associated oxidative damage. From a mechanistic standpoint, *O. sanctum* contributes significant antioxidant, anti-inflammatory, and insulin-sensitizing effects that complement and amplify metformin's inhibition of hepatic gluconeogenesis and improvement of peripheral glucose uptake. Together, these agents reinforce key nodes in the insulin signaling cascade, including improved IRS/PI3K/Akt signaling and GLUT4 translocation, while simultaneously reducing reactive oxygen species and lipid peroxidation that otherwise perpetuate insulin resistance. Such multi-targeted modulation is particularly valuable in a disorder as multifactorial as T2DM, where monotherapy frequently fails to sustain long-term metabolic control.

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