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Integrative Analysis of Myrcene's Lipase Inhibition and Anti-Inflammatory Mechanisms in Human Monocytic Cells

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ABSTRACT: Myrcene (MCN), a monoterpene commonly present in essential oils of aromatic plants, was investigated for its dual role in modulating lipid metabolism and inflammation in human monocytic THP-1 cells. MCN was first evaluated for its ability to inhibit pancreatic lipase activity, followed by cytotoxicity screening to determine non-toxic working concentrations. Its anti-inflammatory effects were assessed by measuring TNF-α gene expression and secretion in LPS-stimulated cells using qRT-PCR and ELISA, while NF-ϰB p65 nuclear translocation was examined through immunofluorescence. The influence of MCN on macrophage differentiation was further determined in PMA-treated THP-1 cells. MCN significantly reduced pancreatic lipase activity and suppressed inflammatory responses by downregulating TNF-α, TLR2, and MIP-1, alongside preventing NF-ϰB p65 nuclear migration. Molecular docking supported these findings by confirming favorable interactions of MCN with the catalytic pocket of lipase and the nuclear localization region of NF-ϰB. Collectively, this study identifies MCN as a promising multitarget natural compound with potential therapeutic application in metabolic and inflammation-associated disorders.

1. INTRODUCTION

Inflammation is a fundamental biological response that protects the body against harmful stimuli, including pathogens, tissue injury, allergens, and metabolic stress. While acute inflammation is essential for maintaining tissue homeostasis and promoting repair, persistent or uncontrolled inflammatory responses contribute to the development of several chronic disorders, such as rheumatoid arthritis, atherosclerosis, obesity-associated complications, and other metabolic diseases [1,2]. The inflammatory process is orchestrated by a network of cytokines and chemokines that regulate immune cell recruitment, activation, and communication. Among these mediators, tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin-6 (IL-6) play central roles in amplifying inflammatory signaling and sustaining tissue damage during chronic inflammation [3,4]. However, uncontrolled or persistent release of cytokines and chemokines can drive chronic inflammatory responses, leading to tissue damage and driving the onset and progression of inflammatory conditions such as rheumatoid arthritis, sepsis, and atherosclerosis [5-7].

Myrcene is a naturally occurring monoterpene widely found in the essential oils of plants such as lemongrass, hops, thyme, and bay. It is well known for its broad range of pharmacological activities, especially its pronounced anti-inflammatory effects. Numerous in vitro and in vivo investigations have demonstrated that myrcene regulates inflammatory processes by influencing key molecular targets and signaling pathways. It significantly decreases the levels of pro-inflammatory cytokines, including tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β), and interleukin-6 (IL-6), while simultaneously downregulating the expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), enzymes that play essential roles in the generation of inflammatory mediators [8,9].

In mouse models of colitis induced by dextran sulfate sodium (DSS), β-myrcene treatment markedly attenuated intestinal inflammation by reducing disease severity, preserving tissue integrity, and limiting myeloperoxidase activity. Further mechanistic

analyses indicated that these protective effects were accomplished through inhibition of NF-ϰB p65 nuclear translocation and suppression of mitogen-activated protein kinase (MAPK) signaling pathways, including ERK, JNK, and p38 [8]. In systemic inflammatory conditions, such as diabetic nephropathy and adrenalectomy-induced oxidative stress, myrcene demonstrated significant antioxidant and anti-inflammatory benefits by restoring the functions of major antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) and by reducing lipid peroxidation marker malondialdehyde (MDA), thereby protecting tissues from oxidative injury [10,11].

Additionally, cell-based investigations in human chondrocytes have demonstrated that myrcene reduces interleukin-1β–driven inflammatory responses by lowering nitric oxide production and reducing the expression of matrix metalloproteinases MMP-1 and MMP-13, which play key roles in cartilage breakdown and joint inflammation [12]. Collectively, these findings suggest that β-myrcene mediates its anti-inflammatory effects through multiple interconnected mechanisms, including suppression of pro-inflammatory mediators, inhibition of NF-ϰB and MAPK signaling pathways, and enhancement of cellular antioxidant defense systems. This wide range of biological activities highlights the potential of myrcene as a promising natural compound for developing anti-inflammatory and cytoprotective therapies.

This study aimed to evaluate the lipase inhibitory potential of the compound and to assess its anti-inflammatory activity in human monocytic THP-1 cells using in vitro experimental approaches.

2. MATERIALS AND METHODS

2.1 Chemicals

Myrcene, porcine pancreatic lipase, orlistat, 4-nitrophenyl octanoate, DEPC-treated water, Triton X-100, chloroform, isopropanol, dimethyl sulfoxide (DMSO), potassium phosphate monobasic, and phorbol 12-myristate 13-acetate (PMA) were procured from Sigma-Aldrich (Germany). RPMI-1640 medium, fetal bovine serum (FBS), and penicillin-streptomycin solution were obtained from Gibco (USA). MTT reagent, TRIzol reagent, DAPI, Alexa Fluor 594 goat anti-rabbit IgG (H+L), and other fluorescence-related reagents were purchased from Invitrogen (USA). The iScript cDNA synthesis kit was supplied by Bio-Rad (Hercules, CA, USA), whereas the Power SYBR Green PCR Master Mix was obtained from Applied Biosystems (UK). The NF-ϰB p65 polyclonal antibody was purchased from Thermo Fisher Scientific (USA). Bovine serum albumin (BSA) and p-nitrophenol were acquired from SRL (Mumbai, India). Potassium chloride, sodium chloride, sodium nitrate, and dipotassium hydrogen phosphate were obtained from Merck (Germany). Unless otherwise specified, all chemicals and reagents used in the study were of analytical grade and were purchased from Himedia Laboratories (Mumbai, India).

2.2 Lipase Inhibitory Activity

To determine the lipase inhibitory potential of MCN, porcine pancreas lipase enzyme (1mg/1mL in potassium phosphate buffer, pH 7.0) was used as the source. 4-nitrophenyl octanoate was used as the substrate and was solubilized in 100% absolute ethanol. Orlistat was used as a positive control, and it was dissolved in DMSO. The absorbance was measured at a wavelength of 405 nm, at a temperature of 26°C in a microplate reader (Thermo Fisher Scientific) for every 10 minutes up to 1 hour [13,14].

2.3 Cell Culture

THP-1 human monocytic cells procured from the National Centre for Cell Science (Pune, India) were maintained under standard culture conditions as described previously [5]. Cells were exposed to various concentrations of MCN prior to assessment of viability and inflammatory responses [7]. To induce an inflammatory response, THP-1 cells were stimulated with LPS (0.5 µg/mL) for 3 hours. For differentiation studies, cells were exposed to PMA (25 ng/mL) for 48 hours to promote monocyte-to-macrophage transition, and morphological changes were documented using an inverted microscope.

2.4 Cell Viability- MTT Assay

The effects of different concentrations (36, 72, 108, and 144 µM) of myrcene (MCN) on THP-1 cells were assessed using 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays, following the methodology described by Kumar et al., 2025 [5]. For cytotoxicity assessment, THP-1 cells were plated at a density of 5×10^5 cells/mL and incubated with increasing concentrations of MCN (36-144 µM). Following overnight exposure, cells were washed with RPMI medium and incubated with MTT reagent for 4 h. The resulting formazan crystals were dissolved in DMSO, and absorbance was measured at 570 nm with background correction at 690 nm [15,16].

2.5 Quantification of transcripts of TNF-α, MIP-1, and TLR2

Total cellular RNA was extracted from treated THP-1 cells using TRIzol reagent according to the manufacturer's instructions. First-strand cDNA synthesis was performed using the iScript™ cDNA synthesis kit. Quantitative PCR reactions were carried out using SYBR Green chemistry and gene-specific primers for TNF-α, TLR2, and MIP-1 (Table 1). Amplification specificity was confirmed by melt curve analysis, in which fluorescence was monitored as a function of temperature [5,7,17]. Relative transcript abundance was calculated after normalization against GAPDH expression.

Table 1. Primers used for real-time quantitative PCR assays.

Sl. No.	Genes		Sequences
1	GAPDH	Forward Reverse	5′-CACCAAACTGCTTAGCACCCC-3′ 5′-TGGTCATGAGTCCTTCCACG-3′
2	TNF-α	Forward Reverse	5′-CCCAGGGACCTCTCTCTAATC-3′ 5′-ATGGGCTACAGGCTTGTCACT-3′
3	MIP-1	Forward Reverse	5′-TTGTGATTGTTTGCTCTGAGAGTTC-3′ 5′-CGGTCCTCACCAGACACACT-3′
4	TLR 2	Forward Reverse	5′-GCCAAAGTCTTGATTGATTGG-3′ 5′-TTGAAGTTCTCCAGCTCCTG-3′

2.6 Determination of secreted proinflammatory marker TNF-α by the ELISA method

To quantify secreted TNF-α, culture supernatants from treated THP-1 cells were collected following LPS stimulation [5,7]. Cytokine levels were determined using a commercial human TNF-α ELISA kit according to the supplier's instructions (BD Biosciences, San Diego, CA, USA). Absorbance was recorded at 450 nm with wavelength correction at 570 nm, and cytokine concentrations were calculated from a standard calibration curve.

2.7 Immunofluorescence detection by confocal microscopy

To evaluate NF-ϰB p65 nuclear translocation, treated THP-1 cells were fixed with paraformaldehyde and permeabilized using Triton X-100. Following blocking with 3% BSA, cells were incubated with anti-NF-ϰB p65 primary antibody and subsequently stained with Alexa Fluor 594-labelled secondary antibody. Nuclei were counterstained with DAPI and visualized using confocal microscopy. The intracellular distribution of NF-ϰB p65 was analyzed to assess the effect of MCN on NF-ϰB signaling [16].

2.8 PMA (phorbol-12-myristate-13-acetate) stimulated monocyte-derived macrophage differentiation

THP-1 cells grown in serum-free medium (2% v/v) were pretreated with MCN and stimulated with PMA (25 ng/mL) for 48 h at 37°C in 5% CO_2, as described by Dong et al., 2014 [18]. The phenotype of THP-1 cells was observed under an inverted microscope. THP-1 cells without PMA were used as control cells (undifferentiated).

2.9 Molecular Docking

To investigate the molecular interactions of myrcene with pancreatic lipase and NF-ϰB p65, docking analyses were performed using experimentally determined protein structures obtained from the Protein Data Bank (PDB). The crystal structures of the pancreatic lipase-colipase complex (PDB ID: 1LPB) and NF-ϰB (PDB ID: 1NFI) were selected as target proteins. Structural preprocessing was carried out using PyMOL Molecular Graphics System (Version 3.1.4.1, Schrödinger, LLC). For the NF-ϰB structure, only the p65 subunit (Chain A) was retained, while all other chains and non-essential components were removed.

Similarly, Chain B corresponding to pancreatic lipase was isolated from the lipase-colipase complex and saved as an independent protein structure for subsequent analysis [19].

The molecular structure of the myrcene (3D coordinates) (PubChem CID: 31253) and the orlistat (2D coordinates) (PubChem CID: 3034010) were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Since only the 2D structure/coordinates of orlistat were available in the database, its corresponding 3D structure/coordinates were generated using Open Babel v3.1.1 [20]. Both ligands were prepared for molecular docking using AutoDockTools v1.5.7 [19] separately.

The molecular docking compatible file of orlistat (ligand) and myrcene (ligand) was individually subjected to blind rigid docking with the molecular docking compatible file of lipase (Chain B) (protein) using AutoDock Vina v1.1.2 [21] with ten different random seed values each.

The molecular docking compatible file of myrcene (ligand) was subjected to blind rigid docking with the molecular docking compatible file of NF-κB p65 (Chain A) (protein) using AutoDock Vina v1.1.2 [20] with ten different random seed values.

The molecular docking outputs, including binding affinities and interaction poses, were visualised using the BIOVIA, Dassault Systèmes, Discovery Studio Visualiser, v25.1.0.24284, San Diego: Dassault Systèmes, 2025.

2.10 Statistical Analysis

All measurements were performed in triplicate, and the results are reported as mean values accompanied by standard deviation (SD). Comparisons between experimental groups were carried out using one-way ANOVA to determine statistical significance.

3. RESULTS AND DISCUSSION

3.1 Lipase Inhibitory Effect of MCN

Lipase activity reached its maximum within 20 minutes, as evidenced by an increase in absorbance at 405 nm. Under the same experimental conditions, the standard inhibitor orlistat exhibited 33 ± 2% inhibition observed at 100 μg/mL. The inhibitory potential of MCN against pancreatic lipase was then evaluated under identical conditions. MCN demonstrated a concentration-dependent inhibitory effect, with inhibition rates of 14.2 ± 0.06%, 19.5 ± 0.04%, and 43.6 ± 5% at concentrations of 90, 180, and 360 μM, respectively (Fig 1).

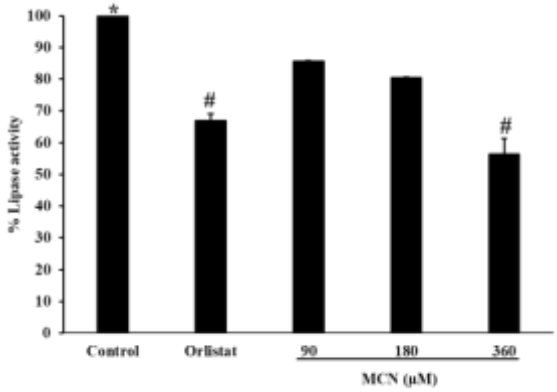


Fig 1. Inhibitory effect of MCN on pancreatic lipase activity. Data represent the mean values obtained from three independent experiments, and error bars indicate standard deviation (SD). Statistical significance was considered at $p < 0.05$.

3.2 Effect of MCN on Cell Viability of THP-1 Cells

The cytotoxicity of the synthesized conjugates on THP-1 cells was determined MTT assay. The THP-1 cells treated with phytocompound MCN at 4 different concentrations (36, 72, 108, and 144 μM) did not have any toxic effects; however, the cell viability was approximately equal to that of the control cells (Fig. 2).

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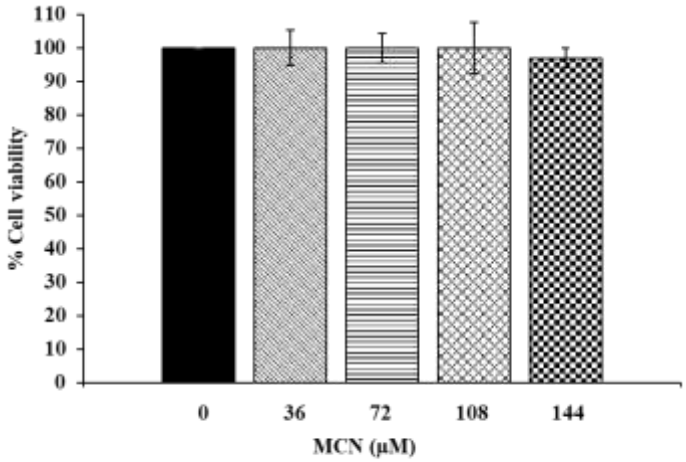


Fig 2. MTT assay showing the effect of MCN on THP-1 cell viability. Cells were treated with varying concentrations of MCN for 15 h at 37 °C in a humidified atmosphere containing 5% CO_2. MCN-treated media without cells served as blank controls. Data are presented as mean ± SD from three independent experiments.

3.3 Effect of MCN on LPS-induced proinflammatory marker in THP-1 cells

The pure metabolite myrcene (MCN) demonstrated a strong inhibitory effect on the expression and a concentration-dependent reduction in the secretion of the pro-inflammatory cytokine TNF-α. When THP-1 cells were treated with increasing concentrations of MCN (36, 72, 108, and 144 µM), a marked reduction in TNF-α transcript levels was observed, showing 1.5 ± 0.07-fold, 80 ± 2.08-fold, 5.5 ± 0.08-fold, and 3.2 ± 0.5-fold decreases, respectively. In contrast, LPS-stimulated control cells exhibited a substantial upregulation of TNF-α expression by approximately 172 ± 4-fold, confirming an active inflammatory response (Fig. 3a).

Furthermore, MCN treatment significantly decreased TNF-α protein secretion in LPS-stimulated THP-1 cells, with the highest concentration (144 µM) reducing TNF-α secretion to 0.88 ± 0.1-fold, whereas the LPS control group showed a 59.8 ± 10-fold increase (Fig. 3b). These findings collectively indicate that MCN effectively suppresses both the transcriptional and protein levels of TNF-α, thereby mitigating excessive cytokine production. These findings indicate that MCN strongly attenuates inflammatory responses by regulating LPS-activated signaling pathways and may contribute to protecting human monocytic cells from inflammation-associated damage.

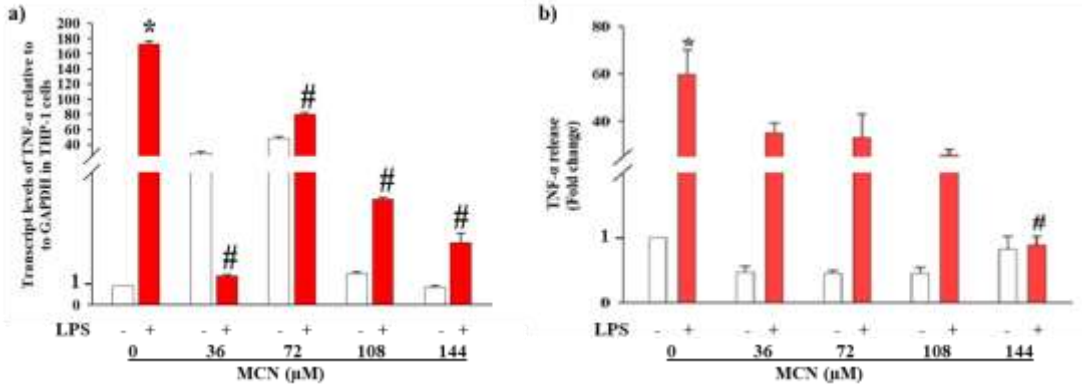


Fig 3. Modulatory effect of MCN on LPS-induced TNF-α production in THP-1 cells. Following MCN pretreatment, cells were challenged with LPS for 3 h. TNF-α transcript levels were determined by qRT-PCR (a), and secreted TNF-α was quantified by ELISA (b). Values represent mean ± SD of three independent experiments. Statistical significance was considered at #$p < 0.001$ *vs* the LPS group and *$p < 0.001$ *vs* the control group.

3.4 Effect of MCN on LPS-activated NF-ϰB Nuclear Translocation

In view of the observed suppression of LPS-induced TNF-α release by MCN, its effect on NF-ϰB (p65) nuclear translocation was further examined in LPS-stimulated human monocytic THP-1 cells. Immunofluorescence analysis revealed that treatment with MCN at concentrations of 108 and 144 μM effectively prevented the migration of the NF-ϰB p65 subunit into the nucleus (Fig. 4b). In LPS-stimulated control cells, the majority of p65 protein was translocated into the nucleus, indicating strong activation of the NF-ϰB pathway (Fig. 4a). However, in MCN-treated cells, p65 remained predominantly localized in the cytoplasm, suggesting that MCN suppresses activation of NF-ϰB through prevention of its translocation to the nucleus and thereby contributes to its anti-inflammatory mechanism.

3.5 Effect of MCN on PMA-driven differentiation of THP-1 monocytes into macrophages

Human monocytic THP-1 cells are spherical cells that grow in suspension and normally do not adhere to the surface of culture plates. Following stimulation with phorbol 12-myristate 13-acetate (PMA, 25 ng/mL) for 48 hours, these cells differentiate into macrophage-like cells, characterized by an adherent morphology and firm attachment to the culture surface (Fig. 5a). To evaluate the impact of MCN on this differentiation process, THP-1 cells were pretreated with varying concentrations of MCN (36-144 μM) for 12 hours, followed by PMA treatment for an additional 48 hours. MCN treatment resulted in a concentration-dependent inhibition of PMA-stimulated differentiation of monocytes into macrophages, with a pronounced suppressive effect observed at 72, 108, and 144 μM (Fig. 5b).

3.6 Modulation of Gene Expression During PMA-induced Differentiation of THP-1 Cells by MCN

PMA stimulation markedly increased the expression of MIP-1 and TLR2 genes in THP-1 cells, with elevations of 17.7 ± 1.2-fold and 2689.04 ± 30.6-fold, respectively, relative to untreated controls. In contrast, pretreatment with MCN significantly reduced this PMA-induced upregulation in a concentration-dependent manner. TLR2 expression was markedly suppressed to 76.3 ± 3.1, 276.1 ± 1.2, 261.1 ± 4, and 246.2 ± 6.8 folds at MCN concentrations of 36, 72, 108, and 144 μM, respectively (Fig. 6b). Similarly, MIP-1 expression was reduced to 2.02 ± 0.3 and 2 ± 0.2 folds at 108 and 144 μM, respectively (Fig. 6a). These findings indicate that MCN effectively downregulates PMA-induced inflammatory gene expression, suggesting its potential role in modulating macrophage activation and attenuating the inflammatory response.

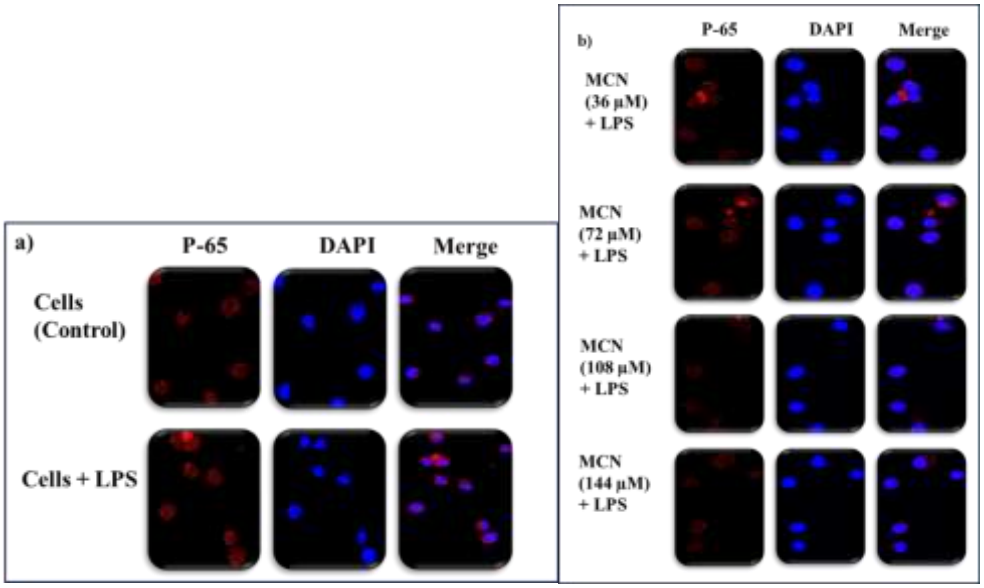


Fig 4. MCN suppresses LPS-induced nuclear translocation of NF-ϰB p65 in THP-1 cells. Cells were stimulated with LPS (0.5 μg/mL), and the cellular localization of NF-ϰB p65 was evaluated by immunofluorescence staining. Alexa Fluor 594 was used to visualize p65, whereas nuclei were identified by DAPI staining. Images were obtained using confocal microscopy and are representative of (a) control cells and (b) cells treated with MCN.

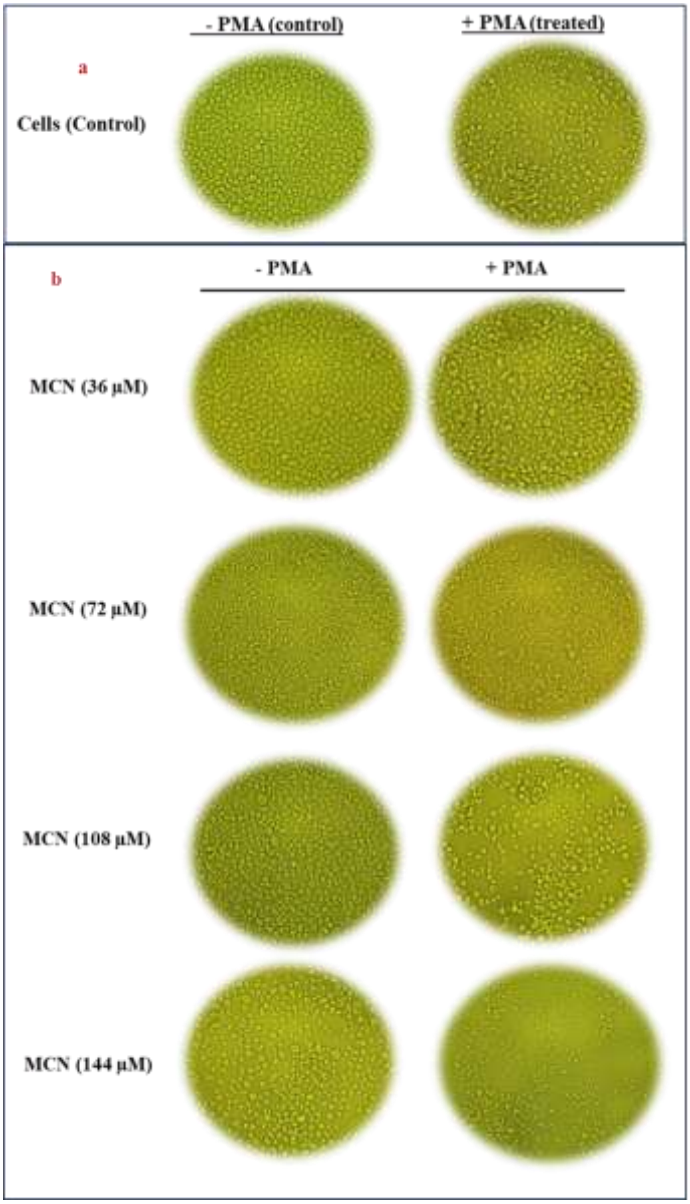


Fig 5. Effect of MCN on PMA-mediated differentiation of THP-1 monocytes into macrophage-like cells. Cells were pretreated with different concentrations of MCN, followed by stimulation with PMA (25 ng/mL) for 48 h. Morphological changes were recorded using an inverted microscope (Magnus). Panels represent: (a) Control and (b) MCN.

3.7 Molecular docking analysis of pancreatic lipase with MCN

Both myrcene and the reference inhibitor orlistat consistently occupied the same active-site pocket of pancreatic lipase (Chain B) in ten independent docking runs, demonstrating the robustness of the docking analysis (Fig. 7a). Orlistat showed the strongest binding affinity (-6.8 kcal/mol), while myrcene displayed a comparable favorable interaction with a binding energy of -5.6 kcal/mol. As reported in Gholami et al. (2025) [22], the catalytic activity of pancreatic lipase is mediated by a conserved catalytic triad comprising Ser152, Asp176, and His263. In the present analysis, orlistat exhibited direct hydrogen bonding interactions with two residues of this catalytic triad, Ser152 and His263 (Fig. 7a and 7b), while myrcene demonstrated a direct interaction with His263 (Fig. 7a and 7c).

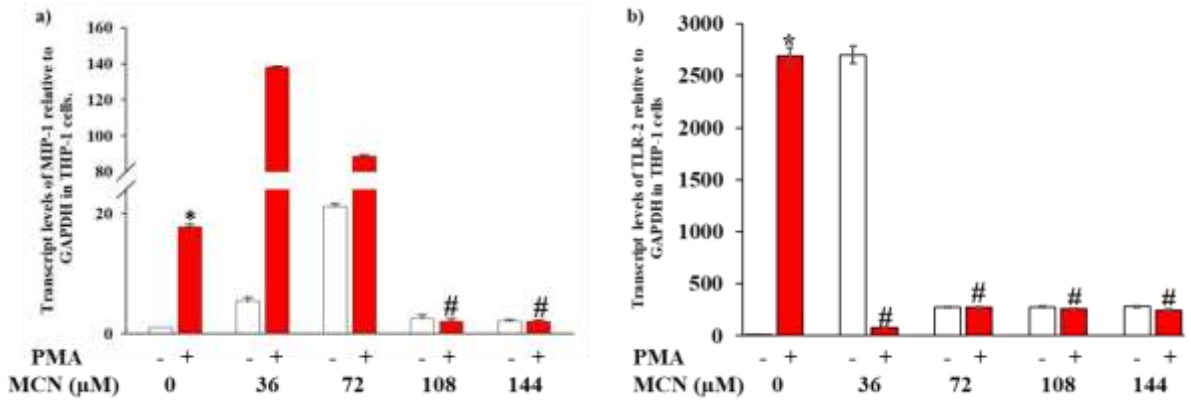


Fig 6. MCN attenuates PMA-induced expression of MIP-1 and TLR2 in THP-1 cells. Following a 12 h pretreatment with MCN, THP-1 cells were stimulated with PMA for 48 h. Relative transcript abundance of MIP-1 (a) and TLR2 (b) was assessed by quantitative real-time PCR. Data are presented as mean ± SD from three independent experiments. Statistical significance was considered at *p < 0.001 versus the untreated control group and #p < 0.001 versus the PMA-treated group.

3.8 Molecular docking analysis of NF-κB with MCN

Across all ten molecular docking analysis performed with different random seeds, myrcene consistently docked in proximity of the nuclear localization signal motif (Lys301, Arg302, Lys303 and Arg304) (Fig. 8a) of NF-κB P65 (Chain A), exhibiting the highest binding affinity of -5.2 kcal/mol. Myrcene directly interacted with an amino acid residue within the nuclear localization signal motif (Arg302) through either alkyl or π-alkyl interaction (Fig. 8b).

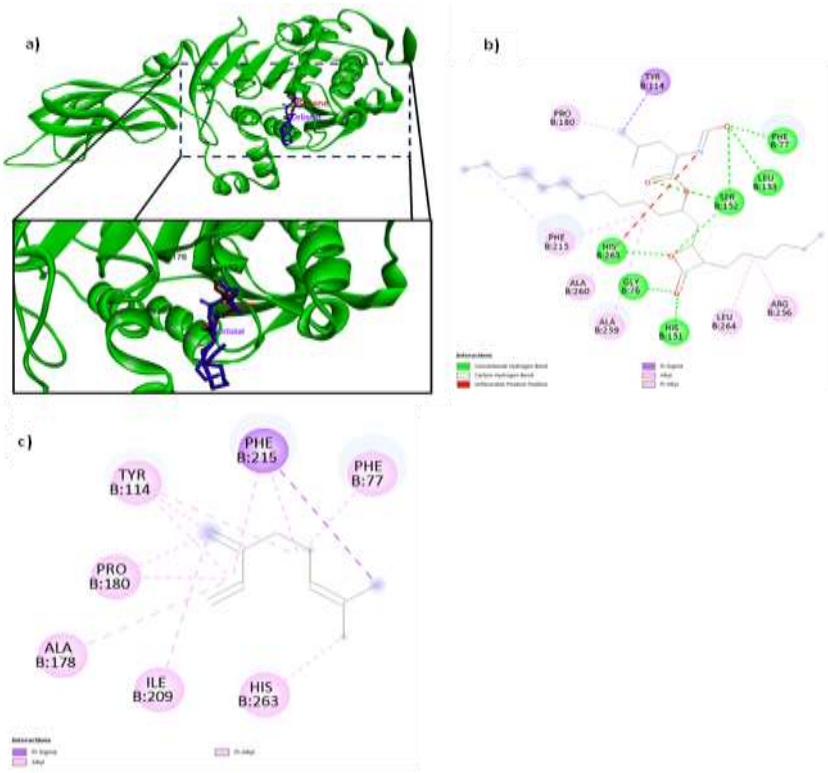


Fig 7. Molecular docking analysis of myrcene (ligand) and orlistat (ligand) on lipase (protein). (a) Binding pocket of myrcene and orlistat on lipase. The catalytic triad (Ser152, Asp176 and His263) are labelled. (b) 2D representation of orlistat (ligand) and lipase (protein) interaction. (c) 2D representation of myrcene (ligand) and lipase (protein) interaction.

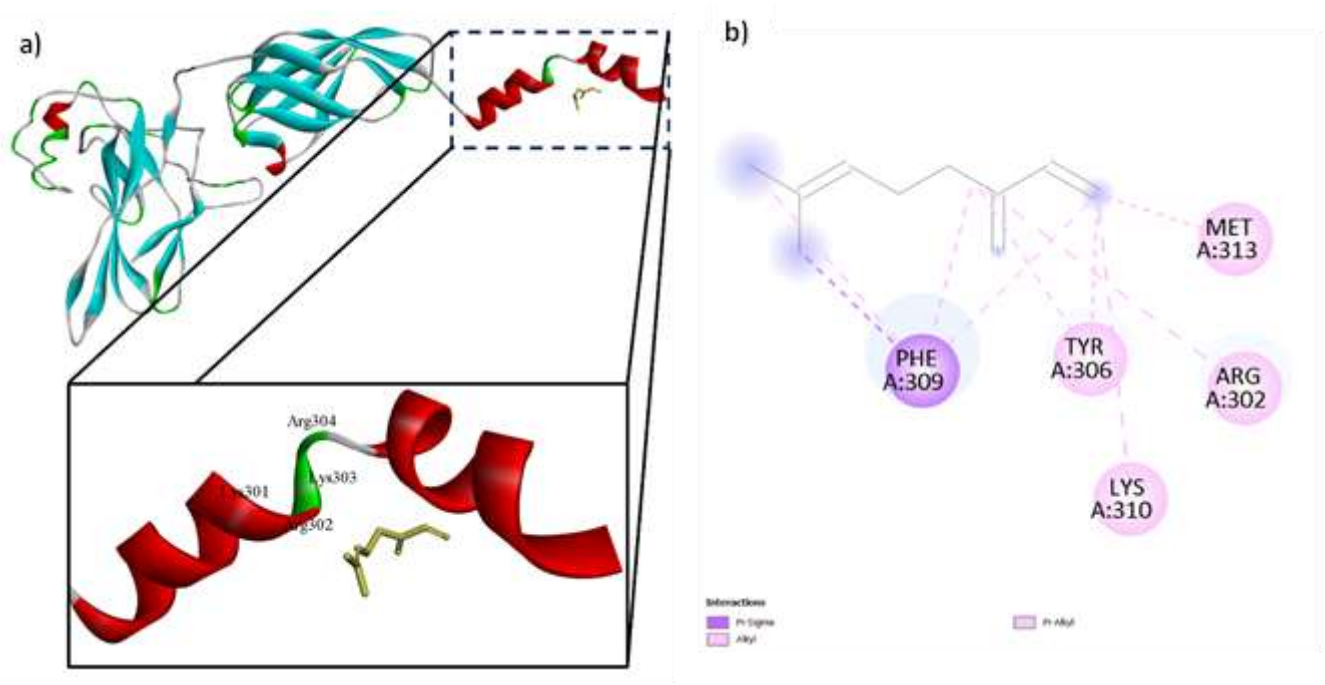


Fig 8. Molecular docking analysis of myrcene (ligand) on NF-κB P65 (protein). (a) Molecular docking of myrcene to the NF-κB P65. The nuclear localization signal motifs (Lys301, Arg302, Lys303, and Arg304) are labelled. (b) 2D representation of myrcene (ligand) and NF-κB P65 (protein) interaction.

3.9 Discussion

The present study demonstrates that myrcene (MCN) possesses both lipase inhibitory and anti-inflammatory activities, suggesting its potential role in regulating interconnected metabolic and inflammatory processes. Using a combination of biochemical, cellular, and molecular approaches, we showed that MCN suppresses pancreatic lipase activity, attenuates inflammatory responses in LPS-stimulated THP-1 cells, and interferes with NF-κB signaling. These findings support the possibility that MCN may function as a multitarget natural compound with relevance to disorders characterized by chronic inflammation and altered lipid metabolism.

Pancreatic lipase is the primary enzyme responsible for the digestion of dietary fats by hydrolyzing triglycerides into absorbable free fatty acids and monoglycerides. Elevated lipase activity can increase circulating free fatty acid (FFA) levels, which in turn stimulate the release of inflammatory mediators, including TNF-α, from monocytes and macrophages [23]. These lipotoxic effects contribute to sustained low-grade inflammation, a defining feature of obesity and metabolic syndrome [24]. The present study revealed that MCN inhibited lipase activity in a concentration-dependent manner, corroborating earlier evidence that plant-derived terpenes and flavonoids act as natural lipase inhibitors [25,26].

Docking analysis further confirmed the molecular basis of this inhibition, with MCN forming interactions at the catalytic triad site (His263) of pancreatic lipase, analogous to orlistat's known binding pattern [22]. Similar observations were made for other terpenoids, such as limonene and pinene, which inhibit pancreatic lipase through hydrophobic and π–π stacking interactions at the catalytic pocket [27]. Thus, MCN may modulate lipid absorption and indirectly reduce inflammatory responses mediated by FFA-induced TLR activation.

Myrcene is well recognized for its potent anti-inflammatory and antioxidant properties [28]. Consistent with these properties, the present study showed that MCN markedly decreased TNF-α expression and secretion in LPS-stimulated THP-1 cells, downregulated TLR2 and MIP-1 gene expression, and suppressed movement of the NF-κB p65 subunit into the nucleus. The anti-inflammatory effects observed in the present study are supported by earlier findings indicating that myrcene can modulate cytokine-mediated inflammatory responses and diminish the expression of major pro-inflammatory markers, including TNF-α, IL-1β, and IL-6, in diverse experimental models [29,30].

Inhibition of NF-ϰB signaling is likely a primary mechanism responsible for the anti-inflammatory activity of MCN. NF-ϰB acts as a critical transcription factor that controls the expression of various inflammatory mediators. Molecular docking analysis indicated that MCN interacts near the nuclear localization signal (NLS) region of the NF-ϰB p65 subunit, particularly at Arg302, which may interfere with nuclear translocation of the NF-ϰB complex and consequently limit the transcription of inflammatory genes. Similar binding behaviour has been reported for other phytochemicals such as curcumin, carvacrol, and α-pinene, which interfere with the NLS region to suppress NF-ϰB activation [31,32]. This interaction underscores the potential of MCN to act as a direct modulator of NF-ϰB signaling at the molecular level.

Computational modelling in this study supports the dual binding affinity of MCN toward both lipase and NF-ϰB targets. MCN exhibited binding affinities of -5.6 kcal/mol with pancreatic lipase and -5.2 kcal/mol with NF-ϰB, suggesting that its moderate yet consistent affinity could be sufficient to modulate biological activity, particularly under physiological accumulation or synergistic phytochemical conditions. Previous molecular docking investigations on other monoterpenes (e.g., menthol, thymol, eucalyptol) also reported comparable binding energies with NF-ϰB and COX-2, which correlate with observed anti-inflammatory outcomes [33-35].

A growing body of literature highlights the intersection between metabolic and inflammatory disorders, where dysregulated lipid metabolism acts as a trigger for chronic inflammation [36]. In this context, natural compounds like myrcene offer therapeutic promise by targeting both processes simultaneously. Studies have demonstrated that terpenoids such as β-caryophyllene and linalool not only inhibit lipase but also suppress NF-ϰB activation and cytokine production, supporting their dual regulatory potential [37-39]. Similarly, in high-fat diet-induced models, dietary terpenes ameliorated lipid accumulation and systemic inflammation through modulation of AMPK and NF-ϰB signaling [40]. These observations align closely with the present findings, indicating that MCN might confer comparable metabolic and anti-inflammatory benefits.

The dual ability of MCN to inhibit lipase activity and suppress inflammatory responses underscores its promise as a potential therapeutic agent for managing metabolic inflammation, obesity, and associated metabolic disorders. Its favorable lipophilicity, natural abundance, and known safety profile enhance its pharmaceutical potential [41]. However, issues related to low aqueous solubility and restricted bioavailability remain to be addressed. Encapsulation in nano emulsions or lipid-based carriers could enhance its therapeutic efficacy, as demonstrated for other terpenes [42]. Furthermore, combinatorial therapy involving MCN and standard anti-inflammatory or lipid-lowering agents may produce synergistic effects with reduced side effects.

4. CONCLUSION

The present study demonstrates that myrcene (MCN), a naturally occurring monoterpene, exhibits dual biological activities by inhibiting pancreatic lipase and suppressing LPS-stimulated inflammatory responses in human monocytic THP-1 cells. The inhibition of lipase activity by MCN suggests its potential role in regulating lipid metabolism and reducing free fatty acid-induced inflammation. Concurrently, its ability to downregulate TNF-α, TLR2, and MIP-1 expression, as well as suppress NF-ϰB p65 nuclear translocation, highlights its strong anti-inflammatory efficacy. Molecular docking studies further supported these findings, revealing that MCN interacts directly with the catalytic triad of lipase and the nuclear localization signal region of NF-κB, indicating a mechanistic basis for its bioactivity. Together, these results suggest that MCN acts as a multi-target modulator that bridges metabolic and inflammatory pathways, offering therapeutic potential against metabolic inflammation, obesity-related disorders, and chronic inflammatory diseases.

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AUTHOR CONTRIBUTIONS

Smruti Ranjan Das: Conceptualization, study design, investigation, and drafting the original manuscript.

Satyabrata Acharya: Methodology, Laboratory Analysis.

Sachidanand Nayak: Methodology, data visualization, and formal analysis.

Asenath Shishak: Methodology, data visualization, and formal analysis.

Aditya Ankari: Formal analysis, review, and editing.

Sarada D. Tetali: Resources, supervision and editing of the manuscript.

CONFLICT OF INTEREST

The authors have no conflicts of interest to disclose.

ETHICS STATEMENT

Ethical approval was not required for this study.

DATA AVAILABILITY STATEMENT

Data supporting the findings of this study are available from the corresponding author upon reasonable request.

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