INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print); 2222-5234 (Online)
Website: https://www.innspub.net
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Vol. 27, Issue: 3, p. 30-41, 2025

RESEARCH PAPER OPEN ACCESS

Exploring the mechanism of 5,7-dimethoxy coumarin in the management of insulin resistance- A network pharmacology and experimental approach

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Key words: 5,7 Dimethoxy coumarin, Insulin resistance, Network pharmacology, Molecular docking, Oxidative stress

DOI: https://dx.doi.org/10.12692/ijb/27.3.30-41 Published: September 06, 2025

ABSTRACT

Insulin resistance (IR) is a pathological condition that plays a central role in the onset of type 2 diabetes mellitus and other related metabolic disorders. The present study explored the therapeutic potential of 5,7 Dimethoxy coumarin (5,7 DMC) against IR utilizing a network pharmacology and *in vitro* approaches. From the targets of 5,7 DMC and IR, 53 intersecting targets were identified. Network analysis identified TNF, NRF2, MAPK1, JAK1, GSTP1, AKT1, MTOR, FOS, PPARA and NFKBIA as hub genes. According to Gene Ontology and Kyoto Encyclopedia of Genes and Genome pathway enrichment analysis, these targets were primarily associated with insulin signaling, oxidative stress and inflammatory pathways. Furthermore, ADME profiling, indicated favourable pharmacokinetic characteristics, including a logP of 1.92, TPSA of 48.67 A^2 and non-hepatoxic nature suggesting good oral bioavailability and cardiac safety of 5,7 DMC. Molecular docking studies confirmed high affinity interactions between 5,7 DMC with major target proteins, supporting its multi-target potential. *In vitro* experiments on high glucose induced insulin resistant-3T3-L1 adipocytes demonstrated that 5,7 DMC significantly improved the activities of antioxidant enzymes (SOD, CAT, GPx, GR, GST) and GSH levels with a decrease in lipid peroxidation markers. Further, it decreased the levels of proinflammatory cytokines (TNF-α, IL-6 and IL-1β) confirming its antioxidant and anti-inflammatory properties. Overall, the results suggest that 5,7 DMC is a potential therapeutic candidate for insulin resistance and its associated complications.

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INTRODUCTION

Insulin resistance (IR) is a pathological condition in which target tissues show diminished response to insulin, causing persistent hyperglycaemia that frequently precedes the development of diabetes mellitus (Zhao *et al.*, 2023). The onset of IR involves an interplay of genetic and environmental factors, with obesity plays a key role in its progression. As IR progresses, the uptake of glucose by skeletal muscle and adipose tissue declines, leading to the development of type 2 diabetes. This dysfunction prompts compensatory hyper insulinemia, which eventually leads to the exhaustion of pancreatic β-cells and a decline in insulin secretion (Krause and De Vito, 2023). While the exact sequence of molecular events leading to IR is still being studied, an in-depth understanding of its mechanisms is crucial for designing effective therapeutic strategies to manage diabetes.

Network pharmacology is an emerging interdisciplinary field that provides a comprehensive perspective on drug mechanisms and their therapeutic potentials (Gong *et al.*, 2018) By integrating bioinformatic platforms and databases, this approach shifts the paradigm from the conventional "one-drug, one-target" strategy to a multi-target, systems-level framework. It explores complex interactions among drugs, biological targets and disease pathways, thereby offering novel insights into drug discovery and development (Jayachandran *et al.*, 2021). Phytochemicals have demonstrated promising effects in alleviating IR by modulating signaling pathways associated with nutrient metabolism (Sayem *et al.*, 2018). These naturally derived compounds improve insulin receptor activation and promote glucose absorption in insulin dependent tissues.

5,7-dimethoxy coumarin (5,7-DMC) is a benzopyrone derivative present in *Citrus aurantifolia*, *Citrus limon* and *Citrus bergamia*. The methoxy substituents at the 5 and 7 positions enhance its biological activity, contributing to its reported anti-inflammatory, antioxidant, anticancer and anti-diabetic properties (Usman *et al.,* 2023). Previous research has shown that 5,7-DMC has the ability to regulate key cellular pathways involved in various metabolic disorders. The present study is designed to identify the potential molecular targets of 5,7-DMC in IR using a network pharmacology approach, as well as to evaluate its antioxidant and anti-inflammatory effects in insulin-resistant 3T3-L1 cells.

MATERIALS AND METHODS

Retrieval and analysis of physicochemical properties of 5,7-DMC

The three-dimensional structure of 5,7-DMC was retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/), and its physicochemical properties were obtained using SwissADME (http://www.swissadme.ch/) and Molinspiration databases (Noor, 2022).

Retrieval of putative targets of 5,7-DMC

To determine the potential molecular targets of 5,7-DMC, multiple target prediction tools were employed. The databases employed are Swiss Target Prediction (http://www.swisstargetprediction.ch/), Pharm Mapper (http://www.lilab-ecust.cn/ pharm-mapper/submitfile.html), SuperPred (https://prediction.charite.de/subpages/target_ prediction) and the Comparative Toxicogenomic Database (https://ctdbase.org/). Redundant targets were eliminated (Wei *et al.*, 2022).

Identification of disease targets: Insulin resistance

Targets related to "insulin resistance" were obtained from publicly available databases including DisGeNET (https://www.disgenet.org/), OMIM (https://www.omim.org/), and GeneCards (https://www.genecards.org/). The collected protein names were then mapped to their respective gene symbols using the UniProt database. To create a non-redundant and comprehensive list of insulin resistance-associated targets duplicate entries were removed (Zhou *et al.*, 2021).

Acquisition of intersection targets

To identify shared targets between 5,7-DMC and IR, the respective target datasets were uploaded to Venny

2.1.0 (https://bioinfogp.cnb.csic.es/tools/venny/). This tool enabled the visualization and identification of overlapping targets (Wei *et al.,* 2022).

Protein–Protein interaction (PPI) network

The overlapping targets identified through Venny 2.1.0 were subsequently imported into the STRING 11.0 database (https://string-db.org/) for the construction of a protein–protein interaction (PPI) network. To maintain biological relevance and species specificity, the analysis was restricted to *Homo sapiens*, and confidence core seat at 0.400 (highest confidence) (Cao *et al.*, 2021).

Visualization of molecular interaction and screening of hub genes

To map the molecular interaction network and identify key hub genes, protein–protein interaction (PPI) data from the STRING database were imported into Cytoscape software (version 3.9.1). In the constructed network, 5,7-DMC was defined as the 'source node,' and the 53 overlapping genes were labelled as 'target nodes.' The Cytohubba plug-in was used to determine crucial hub genes by evaluating their connectivity and topological properties within the network. Additionally, the MCODE (Molecular Complex Detection) plug-in was employed to identify highly interconnected clusters, from which the top-scoring core targets were selected for molecular docking (Cheng *et al.*, 2021).

GO and KEGG pathway enrichment analysis

The 53 overlapping genes were subjected to Gene Ontology (GO) was carried out with the target's gene using David online tool (Lin *et al.*, 2014). This analysis categorized the targets into biological processes (BP), cellular components (CC), molecular functions (MF), and signaling pathways. GO terms with a p-value less than 0.05 were considered statistically significant, while a stricter cutoff of $p < 0.01$ was applied for selecting KEGG pathways. The most significant GO terms and pathways were then used to highlight the potential biological mechanisms by which 5, 7-DMC may exert therapeutic effects against IR.

Network construction

Several interaction networks, with compound, target, pathway were generated by using the Cytoscape platform (Yen *et al.*, 2011).

Molecular docking

Docking studies were performed on the chosen hub genes using PyRx software. The structures of the target proteins obtained from the Protein Data Bank (PDB), were docked with 5,7-DMC. The resulting binding affinities and interactions were analyzed to assess the strength and stability of the compound–protein complexes (Vidhya Rekha *et al.*, 2022).

***In Vitro* studies**

Chemicals

5,7-Dimethoxy coumarin, Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), insulin, antibiotic–antimycotic solution, and 3-isobutyl-1-methylxanthine (IBMX) were procured from Sigma Aldrich Pvt. Ltd., India. All other chemicals and reagents utilized in the study were of analytical grade.

Cell culture and differentiation

3T3-L1 preadipocytes obtained from NCCS, Pune, India, were maintained at 37°C in a 5% CO_2 incubator. They were cultured in DMEM with normal glucose, supplemented with 10% FBS, 100 units/mL penicillin, and 100 µg/mL streptomycin. After reaching confluence, the cells were induced to differentiate by using a medium containing dexamethasone (1µM), IBMX (0.5mM), and insulin (1µg/mL) in DMEM supplemented with 10% FBS. The differentiation medium was changed every two days until the formation of mature adipocytes (Kim *et al.*, 2009).

Induction of insulin resistance

To induce IR, 3T3-L1 adipocytes were treated with glucose (25mM) and insulin (0.6nmol/L) for 24 hours. The development of insulin resistance was confirmed by evaluating the residual glucose levels in the media across all experimental groups.

Experimental groups

The experiments were carried out in four groups: Group I: Normal control (3T3-L1 adipocytes) Group II: Diabetic control (IR-3T3-L1) (Choi *et al.*, 2017) Group III: Diabetic cells treated with 5,7-DMC (60 µM) Group IV: Diabetic cells treated with rosiglitazone (0.1 µM) (Gao *et al.*, 2015)

Measurement of antioxidants

The activities of superoxide dismutase (SOD) (Kakkar *et al.*, 1984), catalase (CAT) (Sinha *et al.*, 1972), glutathione peroxidase (GPX) (Rotruck *et al.*, 1973), glutathione reductase (GR) (Pinto and Harley, 1978), glutathione-S-transferase (GST) (Habig *et al.*, 1974), and were measured in the cell lysates of the experimental groups. Additionally, the levels of reduced glutathione (GSH) (Ellman, 1959) and thiobarbituric acid reactive substances (TBARS) (Niehaus and Samuelson, 1968) were quantified.

Estimation of inflammatory markers

Inflammatory markers TNF-α, IL-1β, and IL-6, were analysed using ELISA kits (Bio Legend, CA, USA) as per manufacturer's information (Niehaus *et al.*, 1968).

Statistical analysis

Data are expressed as mean ± standard deviation based on three independent experiments. Statistical analysis was performed using one-way ANOVA, with significance defined at $p < 0.05$. All analyses were conducted using SPSS software version 16.0 (Yen *et al.*, 2011).

RESULTS

3D Structure and physicochemical properties of 5,7-DMC

The 3D structure and physicochemical characteristics of 5,7-DMC are illustrated in Fig. 1 and summarized in Table 1, respectively.

Targets of 5,7-DMC

A total of 615 potential targets for 5,7-DMC were retrieved from the Swiss target prediction (102), SuprePred (354) and Pharm mapper (159) databases. After eliminating duplicates, 143 unique targets were selected for further investigation.

Table 1. Physicochemical and pharmacokinetics properties of 5,7 DMC

Properties	Value
CID	2775
Molecular weight	206.19
Lipophilicity (Log Po/W)	1.92
$CaCO_2$	22.30
BBB permeability	0.154
CNS permeability	-2.37
Hepato toxicity	No
hERG inhibitor	No
Lipinski	Yes
TPSA (A)	48.67 A°

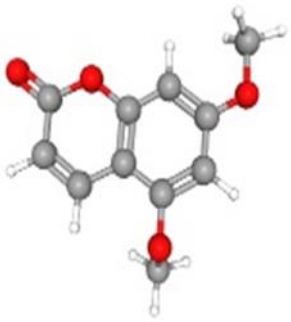

Fig. 1. 3D structure of 5,7 DMC

Targets of insulin resistance

A total of 348, 304, and 2546 insulin resistance-related targets were retrieved from DisGeNET, OMIM, and GeneCards databases, respectively. After removing redundancy, 895 distinct therapeutic targets associated with insulin resistance were consolidated.

Acquisition of intersection targets between 5,7-DMC and IR

The 53 common targets shared by 5,7-DMC and insulin resistance are illustrated in Fig. 2.

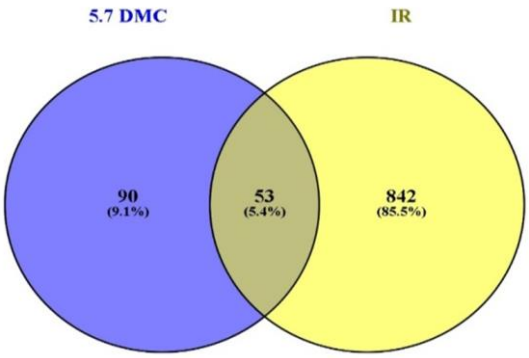

Fig. 2. Intersecting targets of 5,7 DMC & IR

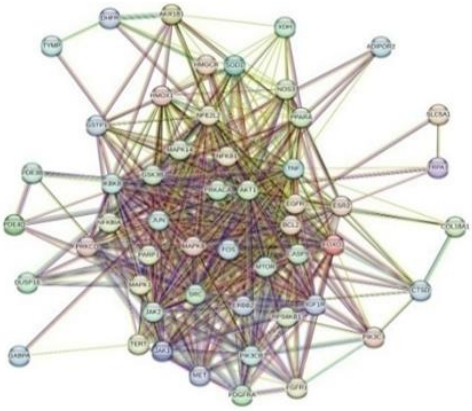

Fig. 3. PPI network of 5,7 DMC and IR

Visualization and analysis of the PPI network

The protein-protein interaction network included 53 nodes representing proteins and 552 edges indicating their interactions using STRING database The network showed an average node degree of 20.8 and a PPI enrichment *p*-value of <1.0e-16, confirming the significance of the observed interactions. The average local clustering coefficient was 0.67 (Fig. 3). Proteins with higher degree values are considered to play more central roles within the network.

Screening of hub genes

Based on the betweenness, closeness, average shortest pathlength, and rank among 14 core targets from PPI network, top 10 hub genes TNF, NRF2, MAPK1, JAK1, GSTP1, AKT1, MTOR, FOS, PPARA, and NFKBIA were retrieved by using various plug ins in cytoscape and are shown in (Fig. 4).

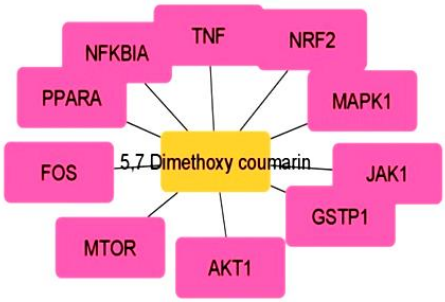

Fig. 4. Hub genes

GO and KEGG pathway enrichment analyses

The analysis of biological processes indicated that the common targets were mainly enriched in pathways involving protein phosphorylation, activation of MAPK and JNK cascades, phosphatidylinositol-mediated signalling, and insulin response. Cellular component analysis showed that these targets were predominantly located in the receptor complex, mitochondrial outer membrane, and cell surface. In molecular functions, the targets were chiefly related to AMP-activated protein kinase activity, platelet-derived growth factor-β receptor activity, phosphatidylinositol 3-kinase binding, and insulin receptor binding, as shown in Fig. 5 and 6.

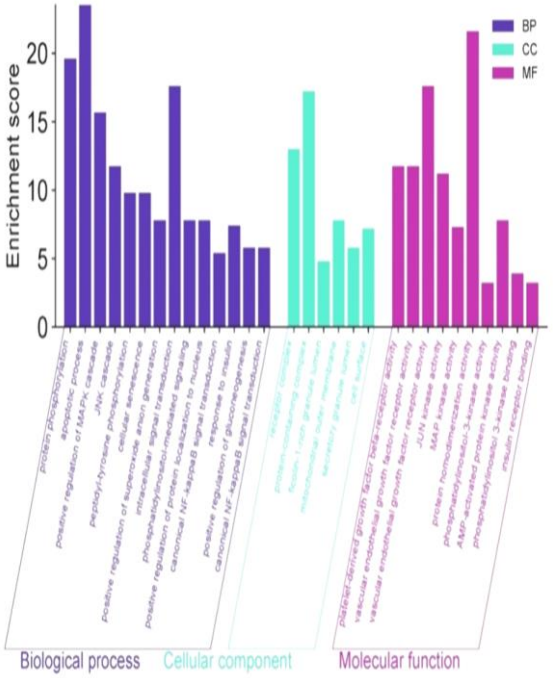

Fig. 5. Gene Ontology of 5,7 DMC

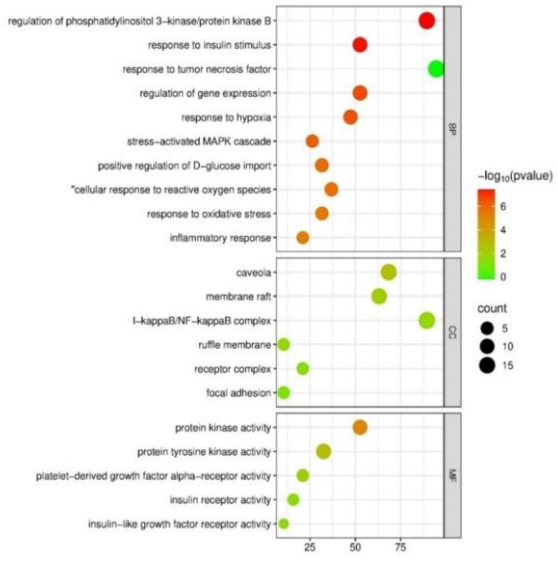

Fig. 6. KEGG enrichment analysis of 5,7 DMC

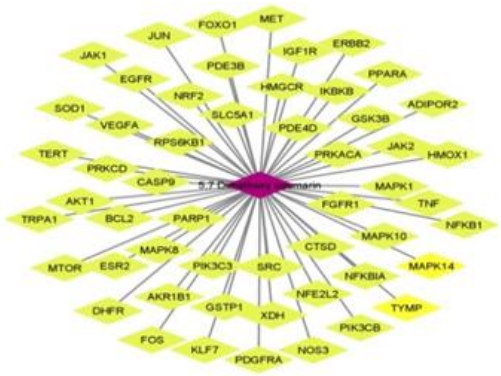

Fig. 7. Compound-Target network

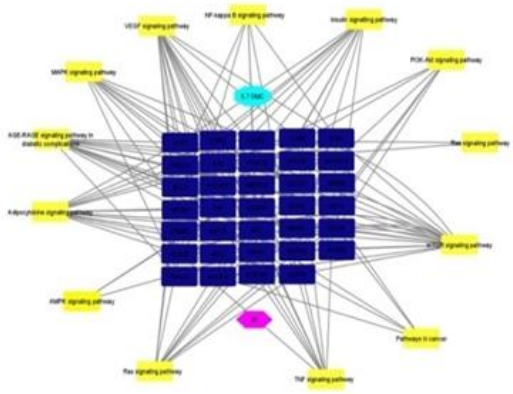

Fig. 8. Target-Pathway network

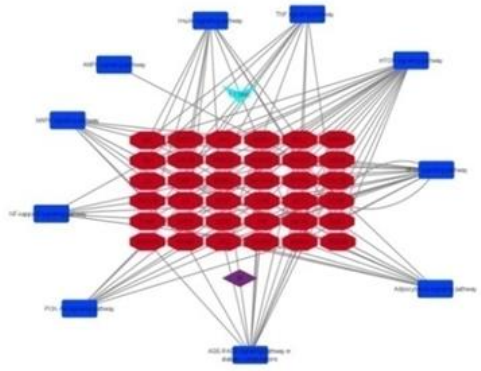

Fig. 9. Compound-Target-Pathway network

Visualization of networks

The C-T, T-P, and C-T-P networks were visualized using Cytoscape 3.9.1, and the resulting network diagrams are presented in Fig. 7 to 9.

Docking analysis

Docking studies with the selected core targets AKT1, TNF, NFKB, and PPARA, mTOR, and Keap-1 was performed with 5,7 DMC (Table 2; Fig. 10 a, b, c, d, e, f).

Table 2. Binding energies of 5.7 DMC with target proteins

Target proteins	PDB ID	ΔG kcal/mol	Interacting residues
AKT1	2UZR	-5.4	Lys17, Tyr18, Arg23, Arg 25, Lys14, Asn53
TNF	2TNF	-7.0	Val17, His 20, Ser 147, Val 150, Gln 149
PPARA	2ZNN	-6.9	Thr 283, Thr 279, Glu 282, Tyr 334, Met 220, As 219, Leu 331, Gly 335, Leu 321, Val 324, Met 320, Ile 317
NFKB	4BWN	-4.2	Ala 318, Leu 322, Ala 323, Ard 319, Lys 326, Lys 325, Lys 321
mTOR	3JBZ	-6.4	Glu 2373, Arg 2368, Trp 2545, Asp 2338, His 23340, Gly 2544
Keap-1	5FNR	-6.6	Ser555, Arg 415, Gly 364, Asn 414, Sr 363, Asn 382, Tyr 334, Phe 577, Tyr 572, Ala556, Ser 602

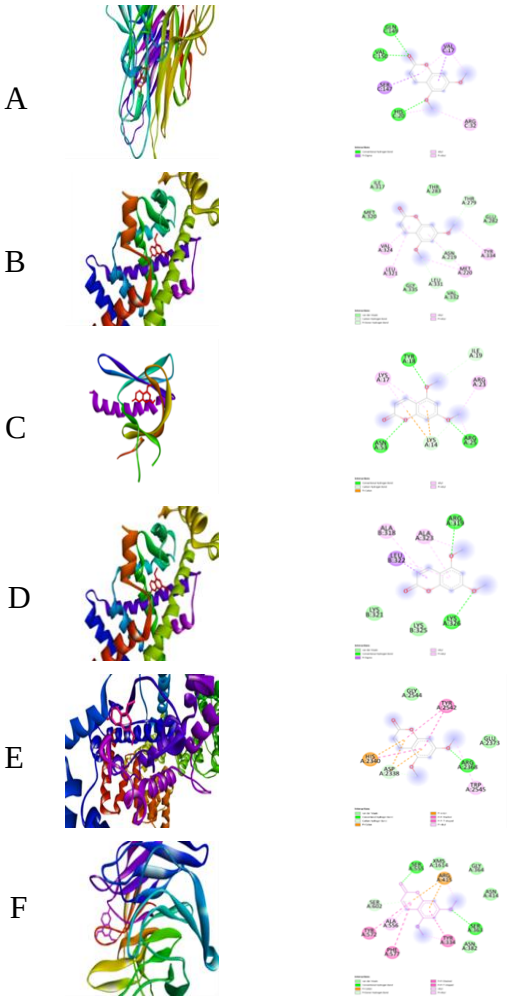

Fig. 10. 2D& 3D interactions of 5,7 DMC with targets
A) TNF B) PPARA C) AKT1
D) NFKB E) mTOR F) Keap-1

Table 3. Influence of 5,7-DMC on oxidative stress markers

Groups	SOD (U/ mg protein)	CAT (U/mg protein)	GPx (nmol/mg protein)	GST (nmol/ mg protein)	GR (U/mg protein)	GSH (nmol/mg protein)
3T3-L1	8.6 ± 0.56^a	5.42 ± 0.33^a	7.31 ± 0.16^a	3.73 ± 0.41^a	8.17 ± 0.07^a	10.33 ± 1.02^a
IR-3T3-L1	3.31 ±0.81^b	1.93 ± 0.08^b	2.12 ± 0.82^b	0.92 ± 0.18^b	5.21 ± 0.14^b	7.61 ± 0.43^b
IR-3T3-L1 +5,7-DMC (60µM)	5.81 ±0.32^c	3.31 ± 0.11^c	5.25 ± 0.78^c	1.83 ± 0.47 ^{c}	6.35 ± 0.39^c	9.06 ± 0.73^c
IR-3T3-L1+ Rosiglitazone (0.1µM)	6.92 ±0.17^d	4.37 ±0.34^d	6.7 ± 0.98^d	2.91 ± 0.02 ^{d}	7.66 ± 0.22 ^{d}	9.16 ±0.59^c

Values represent mean ± standard deviation (SD) based on triplicate experiments (n=3). Statistical differences among the groups were determined using ANOVA, followed by Duncan's post hoc test, with significance considered at $p < 0.05$.

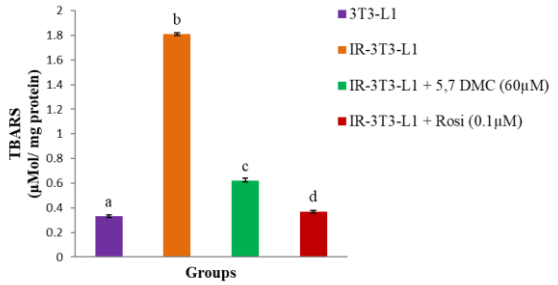

Fig. 11. Effect of 5,7-DMC on TBARS levels in 3T3-L1 cell lines

Values were expressed as mean ± SD (n=3) and the difference between the groups was evaluated by one–way ANOVA followed by Duncan's Post hoc test, p< 0.05.

In Vitro analysis

Influence of 5,7-DMC on oxidative stress markers

A marked decrease in the activity of enzymatic antioxidants SOD, CAT, GPx, GR, GST, and GSH levels was observed in the lysates of experimental cell lines. Treatment with 5,7-DMC(60 µM) for 24 hours significantly restored these antioxidants to near-normal conditions. Likewise, TBARS levels were elevated in IR-3T3-L1 adipocytes compared to the normal control, but were notably reduced following treatment with 5,7-DMC. These results are consistent with the standard anti-diabetic drug rosiglitazone (Table 3; Fig. 11).

Effect of 5,7-DMC on inflammatory cytokines

The levels of inflammatory cytokines (TNF-α, IL-6, IL-1β) were found to be significantly increased in IR-3T3-L1 adipocytes when compared to normal control. Treatment with 5,7-DMC (60µm) for 24 hours, significantly reduced the cytokine levels in IR-3T3-L1 (Fig. 12). Rosiglitazone showed a similar effect.

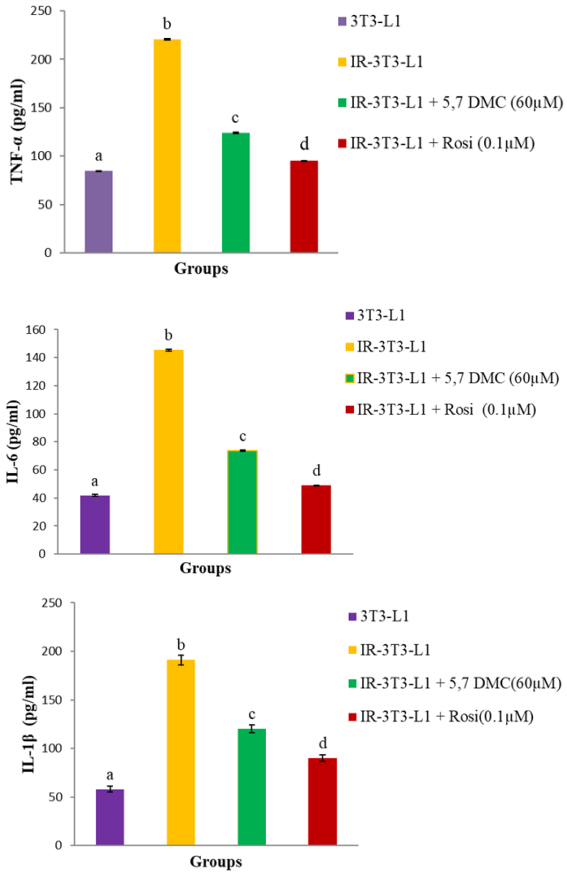

Fig. 12. Effect of 5,7-DMC on inflammatory cytokines levels in 3T3-L1 cell lines

Values were expressed as mean ± SD (n=3) and the difference between the groups was evaluated by one way ANOVA followed by Duncan's Post hoc test, p< 0.05.

DISCUSSION

Insulin resistance characterized by reduced ability of insulin to promote glucose uptake and utilization in peripheral tissues is the central feature of type 2 diabetes mellitus and metabolic syndrome. IR in adipose tissue causes accumulation of fat at ectopic

sites leading to lipotoxicity. Being an active endocrine organ adipose tissue dysfunction leads to impaired insulin signaling in organs such as liver (reducing hepatic insulin sensitivity and enhancing gluconeogenesis), skeletal muscle (impairing glucose uptake), and on the pancreas (leading to β-cell apoptosis) (Hotamisligil *et al.,* 2006).

Phytochemicals have shown significant potential in improving insulin sensitivity by modulating key signaling pathways. However, the clinical applicability of many phytochemicals is limited by their poor pharmacokinetic and pharmacodynamic profiles.

Thus, assessment of ADME properties of the drug is the initial phase in the drug discovery process that provides knowledge on the suitability of a lead (Çevik *et al.,* 2025). It predicts its disposition inside an organism, relating to its pharmacological action. Lipophilicity which determines the ADME and toxicity of the drugs plays a prominant role in the transport of molecules across the membranes. It is considered as the reference parameter to predict the biological activity of drugs candidates (Islam *et al.*, 2024). It has been found out that compounds exhibiting consensus logPo/w in the range of 2.25 to 4.75 have better brain penetration. In the present study, the lipophilicity of 5,7, DMC showed the consensus logPo/w is 1.92, suggesting it as an efficient drug to cross brain barriers that can act on the CNS. LogP that predicts drug-likeness of a new compound is an important component of Lipinski's Rule of Five. Accordingly, drug with LogP value between 1.35-2.5 is an ideal for oral and intestinal absorption (Tsantili-Kakoulidou *et al.*, 2021).

The BBB is a major determinant of drug distribution to the brain. In this study, the BBB permeability of 5,7 DMC is 0.154. The human ether-a-go-go-related gene (hERG) protein is a tetrameric potassium channel that plays an important role in cardiac action potential. Inhibiting the activity of these channels leads to cardiac arrhythmias, and in the present study, 5,7 DMC was found to be a cardio-protective agent. pkCSM is a widely used computational tool that facilitates the prediction of various pharmacokinetic and toxicity profiles, including hepatotoxicity, based on molecular structure and properties (Sang *et al.*, 2024). According to pkCSM predictions, 5,7 DMC does not share structural or physicochemical similarities with known hepatotoxic compounds, and as a result, it is predicted to be non-hepatotoxic.

One of the key descriptors used in predicting pharmacokinetic behavior is the Topological Polar Surface Area (TPSA) which is highly indicative of a compound's absorption, bioavailability, and ability to cross biological value. Generally, a TPSA value of less than 140 $Å^2$ is considered favorable for oral bioavailability. In this study, TPSA of 5,7 DMC was calculated to be 48.6 $Å^2$, suggesting that the compound is highly hydrophobic. This hydrophobicity may enhance its ability to passively diffuse across lipid membranes, thereby facilitating effective membrane permeability. Thus, the physicochemical and pharmacokinetic properties of 5,7 DMC exhibit its efficacy as a potent drug candidate for further studies.

In the present network pharmacological studies, 53 intersecting targets were identified between 5,7 DMC and insulin resistance. The PPI network illustrated that the top10 hub genes were found to be TNF, NRF2, MAPK1, JAK1, GSTP1, AKT1, MTOR, FOS, PPARA and NFKBIA suggesting that inflammation, antioxidant and insulin signaling are the main pathways of action of 5,7 DMC. TNF-α is an important proinflammatory factor that reduces insulin signaling via phosphorylation of serine residues. It plays a vital role in insulin resistance and is capable of increasing free fatty acids in circulation. The down regulation of TNF-α can significantly improve insulin sensitivity in experimental animals (Sethi *et al.*, 2021).

TNF-α is known to activate the MAPK pathway that regulates cellular and biological activities. Akt/mTOR signaling pathway governs multiple cellular functions by activating downstream effector molecules thereby

promoting glucose utilization, protein synthesis, and lipogenesis in adipose tissue (Hoxhaj *et al.,* 2020). Impaired PI3K/Akt pathway leads to insulin resistance that causes accumulation of AGEs, generation of ROS and RNS resulting in diabetic complications (Bhatti *et al.*, 2022).

Nrf2 a basic leucine zipper type of transcription factor is the master regulator that control the expression of antioxidant and phase II detoxification enzymes. Under basal conditions, Nrf2 is sequestered in the cytoplasm targeted for proteasomal degradation by Keap1. However, under chronic diabetic conditions, sustained ERK activation suppresses Nrf2 signaling by markedly inhibiting its expression and translocation that disturbs redox homeostasis cellular resilience (Joof *et al.*, 2025). Thus, activation of the Nrf2 pathway enhances the antioxidative activity to protect cells and maintain cellular homeostatic mechanisms.

The GO enrichment analysis divided into BP, MF, and CC categories were visualized as bubble plot. The size and color represent the number of genes and statistical significance of the enrichment (Zhang *et al.*, 2024). The study indicates that the identified GO term is more strongly associated with IR and revealed that biological process involved were the regulation of phosphatidylinositol 3-kinase/protein kinase B, responses to insulin stimulus, tumor necrosis factor, oxidative stress, inflammatory and apoptotic process. The cellular components were enriched with ficolin-1-rich granule lumen, mitochondrial outer membrane, secretory granule lumen and cell surface. The activities of platelet derived growth factor β-receptor, VEGF receptor, JUN and MAP kinase and insulin receptor were involved in molecular functions. In KEGG enrichment analysis, pathways such as protein phosphorylation, positive regulation MAPK and JNK cascades, phosphatidylinositol mediated signaling, response to insulin and NF-kB mediated signal transduction. The various network interactions highlighted the multifunctional effect of 5,7 DMC in the treatment of IR. In addition, *in silico* docking analysis was carried out with six potential targets using AutoDock version 4.2.6 software. From the binding energies, it is evident that 5,7 DMC efficiently interacted with TNF (-7.0), followed by other targets. From these studies, it is evident that 5,7 DMC interacts with pathways related to antioxidant and inflammation.

Further in vitro studies using 3T3-L1 were carried out to assess the antioxidant and anti-inflammatory potential of 5,7 DMC under high glucose-induced insulin-resistant conditions. In hyperglycemia induced insulin resistant adipocytes, excessive generation of free radicals disrupts its balance with antioxidants causing oxidative stress (Yesupatham and Saraswathy, 2025). This redox imbalance damages lipids, proteins, and nucleic acids, thereby impairing normal cellular signaling. Superoxide dismutase (SOD) is the primary enzymic antioxidant involved in scavenging superoxide anion radicals (O_2^-), converting them into hydrogen peroxide (H_2O_2), which is then further detoxified by catalase (CAT) and glutathione peroxidase (GP_x) (Manzano-Pech *et al.*, 2025). Along with these, glutathione-S-transferase (GST) and glutathione reductase (GR) play vital roles in maintaining the cellular redox cycle. In addition, non-enzymic antioxidants such as reduced glutathione (GSH) act as major free radical scavengers and help preserve redox homeostasis. Hyperglycemia causes abnormal glycation of antioxidant enzymes, resulting in their structural modification and functional decline. This weakens the enzymic defense system, allowing free radicals to attack membrane lipids, initiating lipid peroxidation and producing toxic by-products such as malondialdehyde (MDA) and TBARS, which further damage cellular components and aggravate insulin resistance. Excessive ROS generated under hyperglycemic conditions activate transcription factors, NF-κB and AP-1, which upregulate the expression of pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1β (Michalak, 2025). These cytokines disrupt insulin receptor signaling by promoting serine phosphorylation of insulin receptor substrate (IRS) proteins, thereby impairing downstream pathways like PI3K/Akt.

Investigations revealed that 5,7 DMC at the dose of (60μM) curtailed oxidative stress by enhancing the levels of both enzymic (SOD, CAT, GPx, GSH, GST and GR) and nonenzymic antioxidants (GSH) and decreased the levels of lipid peroxidation markers (TBARS). In addition, the levels of proinflammatory markers were significantly decreased in the cell lysates of 5,7-DMC-treated insulin-resistant 3T3-L1 cell lines. These outcomes are in line with the observations of Liu *et al.* (2023), who reported that chlorogenic acid alleviates oxidative stress and inflammatory responses in metabolically challenged models, thereby supporting its role as a promising therapeutic candidate against insulin resistance.

CONCLUSION

The present study revealed the putative targets of 5,7 DMC in ameliorating insulin resistance observed in type 2 diabetes mellitus. Docking studies showed that 5,7 DMC possessed the highest binding energies with all six targets. Furthermore, *in vitro* studies showed significant antioxidant and anti-inflammatory activities of 5,7 DMC. The study provided a comprehensive understanding of the plausible mechanism of action of 5,7 DMC that may have potential use in the treatment of insulin resistance.

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