doi.org/10.36721/PJPS.2025.38.5.REG.14465.1

Combined computational and pharmacological approach reveals *Citrullus colocynthis* as a natural PCSK9 inhibitor in insulin resistant rats

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Abstract: This study evaluated the therapeutic effects of Citrullus colocynthis seed extract (CCSE) on PCSK9 expression in a high-fat diet (HFD)-induced insulin resistance (IR) model in Wistar rats. Fifteen phytoconstituents from CCSE were screened for drug-likeness using SwissADME and docked against PCSK9 (PDB ID: 6U26). *In vivo*, 30 HFD-induced-IR rats were divided into five groups: one control (saline) and four treatment groups received CCSE (100,200,300,400 mg/kg) for 28 days. Hepatic PCSK9 mRNA expression was analyzed by qRT-PCR, with relative fold changes. Data were statistically evaluated by ANOVA. *In-silico* analysis demonstrated all selected compounds complied with Lipinski's Rule of Five, indicated favorable oral bioavailability. Topological polar surface area (TPSA: 9.23-55.38 Å²) and number of rotatable bonds (NRB <10) further supported their drug-like properties. Strong binding affinities were of compounds Cyclopropane carboxylic acid and 2,4-di-tert-butylphenol (-7.4 and -5.5 kcal/mol) to PCSK9. *In-vivo* results showed that CCSE significantly downregulated hepatic PCSK9 mRNA expression at a dose of 300mg & 400mg/kg ($p < 0.05$ vs. control), with the highest reduction (3.2-fold) observed at the 400 mg/kg dose. CCSE significantly downregulated hepatic PCSK9 mRNA expression and confirmed through both *in-silico* and *In-vivo* approaches. This suggests its potential as a natural therapeutic agent for managing metabolic disorders.

Keywords: PCSK9, *Citrullus colocynthis*, PCSK9 inhibitor, LDL, hypercholesterolemia, insulin resistance

Submitted on 16-04-2025 – Revised on 16-07-2025 – Accepted on 05-05-2025

INTRODUCTION

Citrullus colocynthis (L.) Schrad., commonly known as bitter apple in English, *anaderi* in Sanskrit, *rakhal* in Bengali, *hanzal* in Urdu, *hanjal* in Hindi, *picktumatti* in Tamil and *kattuvellari* in Malayalam, possesses a wide range of medicinal properties. This plant has exhibited antioxidative, hypoglycemic, antibacterial, anticancer, anti-inflammatory, analgesic, gastrointestinal protective, antimicrobial, antidiabetic, hypolipidemic, antineoplastic, profibrinolytic, anti-allergic, pesticidal and immune-stimulatory activities (Li *et al*., 2022). *C. colocynthis* (hereafter abbreviated as) CC contains various bioactive compounds, including cucurbitacins, flavonoids and polyphenols, which contribute to its therapeutic potential (Altemimi *et al*., 2023). Several animal and epidemiological studies have reported the hypolipidemic and hypoglycemic effects of CC (Afshari *et al*., 2021). CC was shown to reduce lipid levels in both animal models and hyperlipidemic human subjects. In clinical trials involving hyperlipidemic non-diabetic individuals, administration of 300 mg/day of powdered CC seeds significantly reduced cholesterol and triglyceride levels. Furthermore, aqueous extracts of CC seeds demonstrated the ability to promote pancreatic beta-cell regeneration and increase pancreatic beta-cell size in rats (Amin *et al*., 2017). The plant's ethanolic extract (1.2 g/kg/day) also normalized blood cholesterol levels in hyperlipidemic rabbits. The pleiotropic effects of CC were comprehensively summarized by Li *et al*., (2022). Hypercholesterolemia include increased level of low density lipoproteins (LDL) resulted from LDL recycling prevention by endocytosis of LDL by lysocomes. Intracellular increased LDL resulted from the binding of low density lipoprotein receptors (LDLR) with proprotein convertase subtilisin/kexin-type 9 (PCSK9) and caused familial hypercholesterolemia. PCSK9 was recognized as a novel therapeutic target for the treatment of hypercholesterolemia (Ahamad & Bhat, 2022). Hypercholesterolemia was associated with increased inflammatory substances leading to altered insulin function and insulin resistance (Hong *et al*., 2022). Insulin resistance and altered metabolic states were reported to affect PCSK9 expression by disrupting lipid homeostasis feedback pathways. Consequently, the complex interactions among a high-fat diet, hepatic insulin resistance and PCSK9 gene expression underscored the nuanced relationships between dietary habits and metabolic dysfunction. Up-regulation of hepatic PCSK9

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mRNA was associated with increased hepatic fat accumulation and elevated cholesterol levels (Emma G. R. *et al*., 2020). In the present study, administration of *Citrullus colocynthis* seed extract (CCSE) significantly may ameliorated these independent risk factors.

Bioactive compounds from medicinal plants played a pivotal role in the development of medicine and healthcare advancements (Asif & Hashmi, 2021; Murthy & Paek, 2021). Therefore, the antihyperlipidemic effects of *Citrullus colocynthis* (CC) bioactive compounds may effect hepatic PCSK9 expression in hypercholesterolemia. This study evaluated the therapeutic potential of *Citrullus colocynthis* seeds extract (CCSE) on PCSK9 hepatic expression in high-fat-diet induced hypercholesterolemic insulin resistant wistar rats.

MATERIALS AND METHODS

In-silico studies

The bioactive compounds of *Citrullus colocynthis* (CC) were retrieved in-*silico* by online searching databases like PubMed, Google Scholar and Web of Science with keywords such as bioactive compounds of *C. colocynthis*, HPLC analysis. GC-MS analysis. Retrieved compounds were labeled (IDs 201-217) for ease and 2D structures were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Drug-likeness and pharmacokinetic properties were evaluated using Swiss ADME following the method described by Chen *et al*., (2020). Compounds showed confirmation to Lipinski's rule of five were selected for molecular docking.

Molecular docking studies

Molecular docking studies of retrieved compounds were performed against PCSK9 (PDB ID: 6U26), as outlined by Mahmoudi Ali *et al*., (2023). Protein preparation steps included removal of attached ligands and water molecules from PDB file, addition of polar hydrogen atoms and repairing missing atoms, assigned charges and PDBQT files were saved for further procedure. Grid construction was carried out in AutoDock Vina with the following dimensions: X = 62, Y = 64, Z = 94; center coordinates: X = 25.691, Y = 10.001, Z = 18.177; spacing: 1.0. Ligand-protein interactions were visualized using Discovery Studio 4.0.

Preparation of animal model

CC seeds were collected from Bahawalpur, Pakistan and authenticated (Voucher No. A144/UCCM). *Citrullus colocynthis* hydro-ethanolic seed extract (CCSE) was prepared according to the protocol by Youl *et al*. (2020). Thirty male Wistar rats (140–180 g, 6–8 weeks old) obtained from UCCM were fed a high-fat diet (HFD) to induce insulin resistance, as described by Zhang *et al*. (2020). Insulin resistance was evaluated through impaired glucose tolerance (IGT) and values of tail vein glucose ranged between 145-199 mg/dl were considered IR. Insulin-resistant rats were randomly divided into five groups containing 6 rats in each group: Group I (normal saline) and Groups II-V, which received CCSE at 100, 200, 300 and 400 mg/kg body weight (Manzoor *et al*.,2022, Sindhu *et al*., 2023) respectively, for 28 days. On day 29, hepatic tissues were harvested for analysis of PCSK9 mRNA expression. Ethical approval for animal handling was granted by departmental review and ethical committee of UCCM, IUB vide No.904/UCCM.

PCSK9 hepatic expression

PCSK9 mRNA expression was analyzed using quantitative real-time PCR (qRT-PCR) based on the protocol of LIU *et al*. (2022). The primer sequences used were: PCSK9 forward: 5′-CACCTTTGGGTCGAGTGCTGAG-3′, reverse: 5′-CGCTGTTGAAGTCGGTGATG-3′; and 18S rRNA forward: 5′-GCACATCGGGGTTGAAGAGG-3′, reverse: 5′-AAACTCTGGGGGAGGTCCGT-3′. GAPDH was used as the reference gene. Fold change in gene expression was calculated using the 2^-(ΔΔCT) method. All experiments were performed in triplicate. Statistical significance was evaluated using Student's *t*-test for individual variables comparing group 1(control) with individual treated groups and one way ANOVA with post-hoc Tuky's test was performed for multiple variables (Group I vs. CCSE-treated groups) and results were expressed as mean ± standard deviation (SD). A *p*-value of <0.05 was considered statistically significant. All test were performed by IBM SPSS V.20.

RESULTS

In-silico analysis retrieved fifty-five compounds from the literature. Among these, the 2D structures of seventeen chemical constituents (IDs 201-217) were available and were subsequently evaluated for drug-likeness and ADMET properties using the SwissADME web-based online portal (table 1). All compounds complied with Lipinski's Rule of Five, which indicated favorable oral bioavailability. Their topological polar surface area (TPSA) values ranged from 9.23 to 55.38 Å², remaining below the 140 Å² threshold, suggested good hydrogen bonding potential and bioavailability. The number of rotatable bonds (NRB) for compounds 201-214 remained below 10, indicated conformational stability. Skin permeability values (Kp: -2.19 to -7.39 cm/s) were low across all compounds, while gastrointestinal (GI) absorption was high for all except compound 210. Blood-brain barrier (BBB) penetration was predicted for most compounds, except 204, 210 and 215-217. Cytochrome P450 (CYP) inhibition varied; compounds 209-212 and 214-217 did not inhibit major CYP isoforms.

However, all compounds-except 206-were predicted to inhibit P-glycoprotein (P-gp). Bioavailability scores were consistently 0.55 for all evaluated compounds. These findings indicated that the selected constituents exhibited

favorable drug-like properties, supporting their potential as candidates for orally administered therapeutics. Detailed results are presented in table 1.

All compounds that met Lipinski's Rule of Five were subjected to molecular docking against the PCSK9 protein (PDB ID: 6U26). The docking analysis revealed that seven compounds (202, 203, 206, 209, 211, 212 and 214) exhibited binding energies indicative of strong affinity toward the 6U26 structure, which corresponds to the C-terminal domain of PCSK9. Cyclopropane carboxylic acid (compound 212) demonstrated the highest binding affinity at -7.4 kcal/mol, while 2,4-di-tert-butylphenol (compound 214) showed the lowest affinity at -5.5 kcal/mol. Results of the molecular docking analysis showed hydrogen bond interactions and the corresponding 2D and 3D molecular structures given in fig. 1.

Based on the docking results, key amino acid residues of PCSK9 namely LEU436, ARG461, VAL435, VAL359, ARG357, ALA328 and ALA649 were found to interact with the seven active CC compounds (202, 203, 206, 209, 211, 212 and 214), suggesting a potential binding site (table 2). The two- and three-dimensional visualizations of the six top-scoring compounds are also illustrated in fig. 1.

Hepatic expression of PCSK9 mRNA and PCSK9-18S mRNA in Group I (insulin-resistant rats) showed relative fold change (2^-ΔΔCT) values of 1.076 ± 0.157 ($P < 0.046$) and 1.038 ± 0.325 ($P < 0.003$), respectively, after normalization to the GAPDH reference gene. Group II exhibited significant downregulation with relative fold change values of 0.662 ± 0.115 ($P < 0.046$) and 0.814 ± 0.252 ($P < 0.003$), respectively.

In Group III, expression levels were reduced to 0.833 ± 0.467 ($P < 0.075$) and 0.893 ± 0.214 ($P < 0.200$), though the changes were statistically insignificant. Group IV demonstrated significant downregulation, with relative fold changes of 0.331 ± 0.054 ($P < 0.001$) for PCSK9 mRNA and 0.206 ± 0.044 ($P < 0.001$) for PCSK9-18S mRNA. Similarly, Group V (treated with CCSE at 400 mg/kg) showed markedly reduced expression values of 0.206 ± 0.044 ($P < 0.026$) and 0.277 ± 0.155 ($P < 0.014$), respectively. Detailed qRT-PCR results, including expression levels of the reference gene (GAPDH) and all treatment groups, are presented in fig. 2 and table 3. Circulatory low density lipoproteins (LDL) in group-I (Mean±SD-mg/dl) was 84.16±3.45 versus in group-II-V was 60.16±5.45, 46.33±3.32, 35.83±1.94 and 31±4.64 (P<0.007) respectively.

DISCUSSION

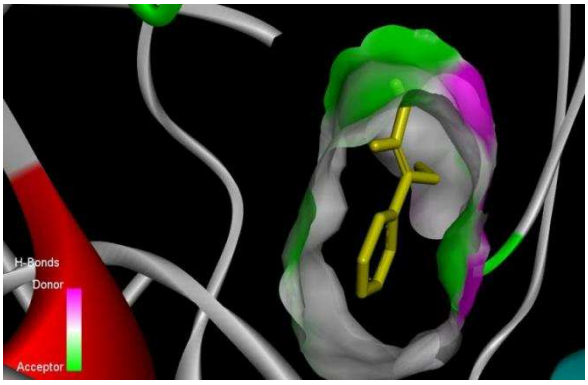
In-silico screening of 55 bioactive compounds from *Citrullus colocynthis* (Hamer & Thamer, 2023; Ahamad & Bhat, 2022) identified 17 potential candidates (IDs 201-217) with favorable drug-likeness and ADMET profiles. These compounds complied with Lipinski's Rule of Five, had acceptable topological polar surface area (TPSA: 9.23-55.38 Å²) and consistent bioavailability scores (0.55). Subsequent molecular docking studies revealed that seven compounds displayed strong binding affinities with the PCSK9 protein (PDB ID: 6U26). Notably, cyclopropane carboxylic acid (compound 212) demonstrated the highest binding affinity (-7.4 kcal/mol).

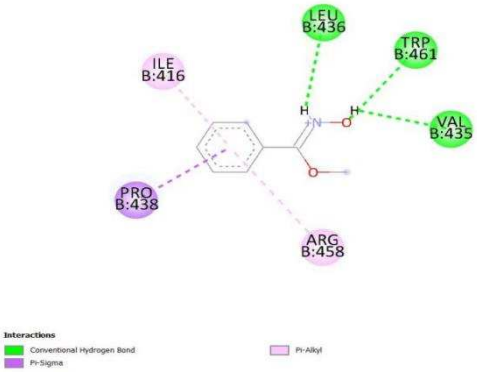
Key interacting amino acid residues included LEU436, ARG461 and ALA328, supporting previously reported interactions (Mahmoudi Ali *et al*., 2023). Structural analysis confirmed that the binding pocket of PCSK9 is located at the interface of the C-terminal domain, allosteric site and catalytic domain (Zainab *et al*., 2021). The C-terminal domain plays a crucial role in conformational alignment and stabilization during PCSK9-LDLR interaction, facilitating lysosomal degradation of LDLR and thus regulating plasma LDL cholesterol levels. Partial deletions in this region (amino acids 457-528 or 608-692) significantly impair PCSK9 secretion (Chapa *et al*., 2023), highlighting its functional importance. In line with this, Iqbal *et al*. (2023) emphasized that the C-terminal domain, in addition to the prodomain, mediates LDL receptor (LDLR) degradation and ligand interaction.

In-vivo studies further validated these findings, where CCSE (*Citrullus colocynthis* seed extract) treatment at higher doses (300 and 400 mg/kg) significantly downregulated hepatic PCSK9 mRNA expression in high-fat diet-induced insulin-resistant rats. The greatest suppression was observed at 400 mg/kg ($P < 0.026$). Expression of both PCSK9 mRNA and PCSK9-18S mRNA was markedly reduced following CCSE administration. These findings are consistent with earlier reports indicating that PCSK9 expression correlates positively with hepatic steatosis and the activation of lipogenic genes such as FAS, APOB and SREBP-1c, which contribute to liver fat buildup.

Transcriptionally, PCSK9 is primarily controlled by regulatory factors including SREBP2, FOXO3, HNF1α and SIRT6. (He Q. *et al*., 2020; Lin *et al*., 2021; Li *et al*., 2022). Downregulation of PCSK9 wass thus emerged as a promising therapeutic target in managing hypercholesterolemia (Ahamad & Bhat, 2022). Clinical studies have also reported that PCSK9 inhibitory therapies (PCSK9-iTs) effectively lower LDL cholesterol levels, consequently reducing the risk of cardiovascular events (CVEs) (Chapa *et al*., 2023). Circulatory low-density lipoproteins (LDL) levels in Group-I were 84.16 ± 3.45 mg/dL, whereas treatment in Groups II to V significantly reduced LDL to 28.5%, 44.9%, 57.4% and 63.2% respectively ($P < 0.007$) (fig. 3). Importantly, PCSK9-mediated pathways are also implicated in insulin resistance.

Oxime methoxyphenyl





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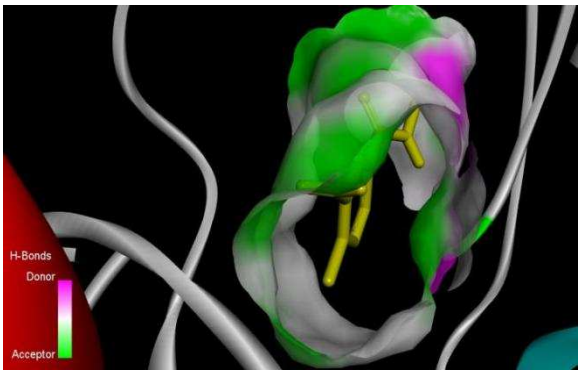
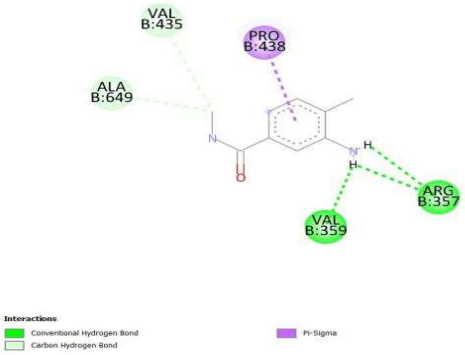
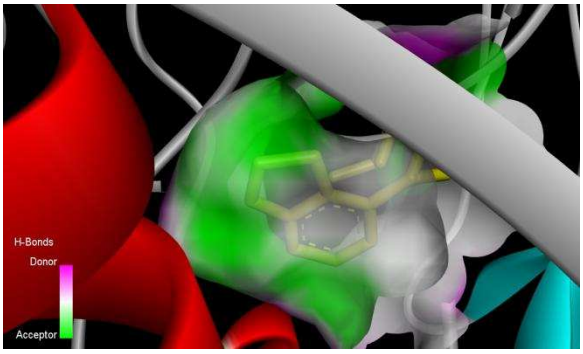
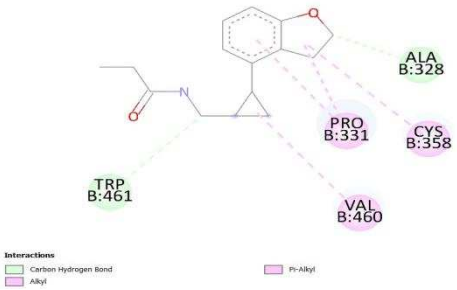


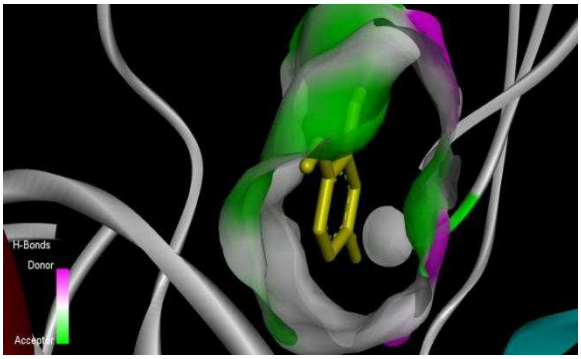


Benzofuran





2 methoxy 4 vinyl phenol



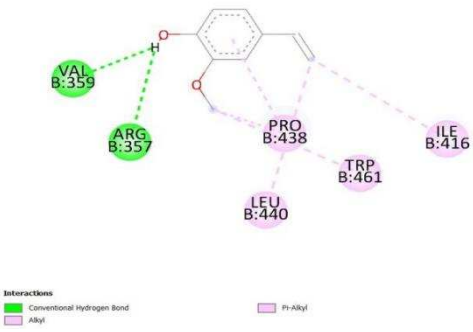
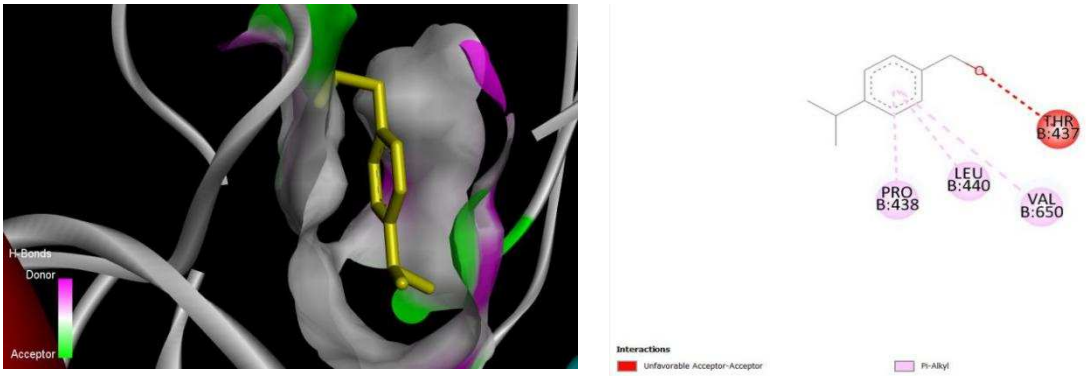
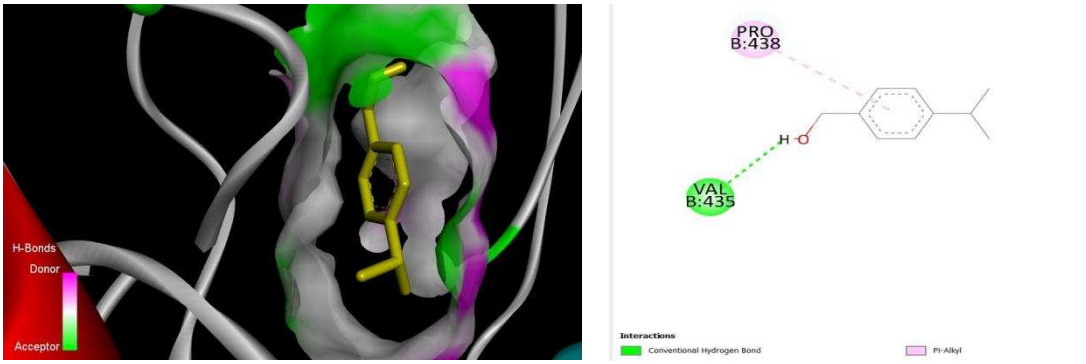


Fig. 1 is continued…

Cymene 7-ol



2-4 Ditert-butylphenol



Cyclopropane carboxylic acid

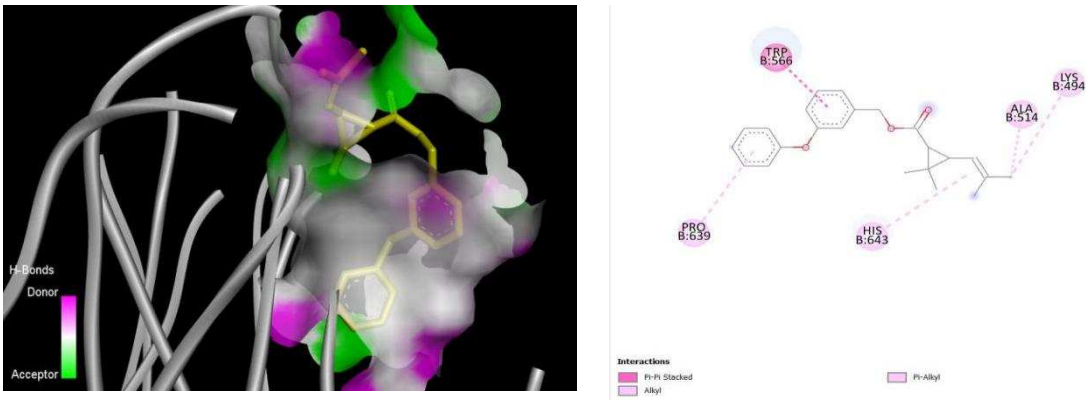


Fig. 1: Molecular docking interactions of CC compounds with 6U26 protein

Free fatty acids and adipokines associated with hypercholesterolemia activate hepatic signaling cascades leading to insulin resistance and reduced pancreatic β-cell volume (Päth *et al*., 2022). PCSK9 itself has been reported to increase insulin granule size and impair insulin function, contributing to insulin resistance (Hong *et al*., 2022). However, PCSK9 downregulation does not impair insulin secretion, indicating its independent regulatory role (Päth *et al*., 2022). Our findings support the dual benefit of CCSE in modulating both lipid and glucose metabolism through its regulatory effect on PCSK9 expression.

CONCLUSION

We showed that CCSE treatment in HFD induced IR rats down regulate transcription of PCSK9 in hepatic tissue. Clinical trials are essential to confirm the potential of C. colocynthis seeds in preventing diabetes and

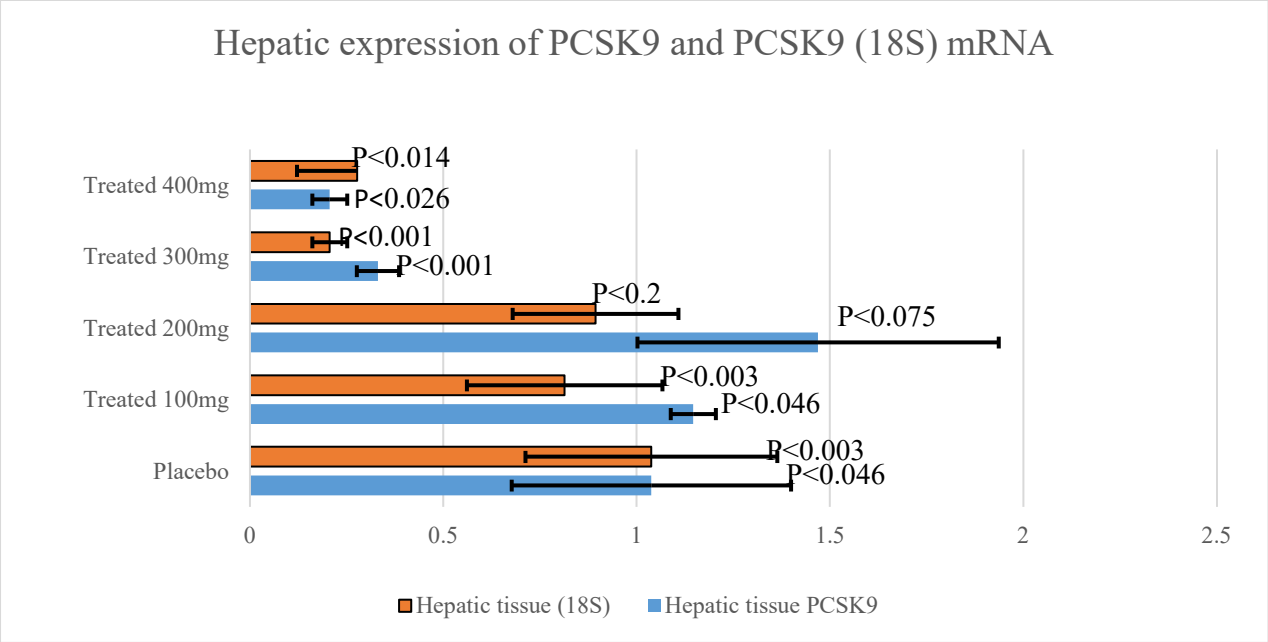


Fig. 2: qRT-PCR results of PCSK9 mRNA hepatic expression

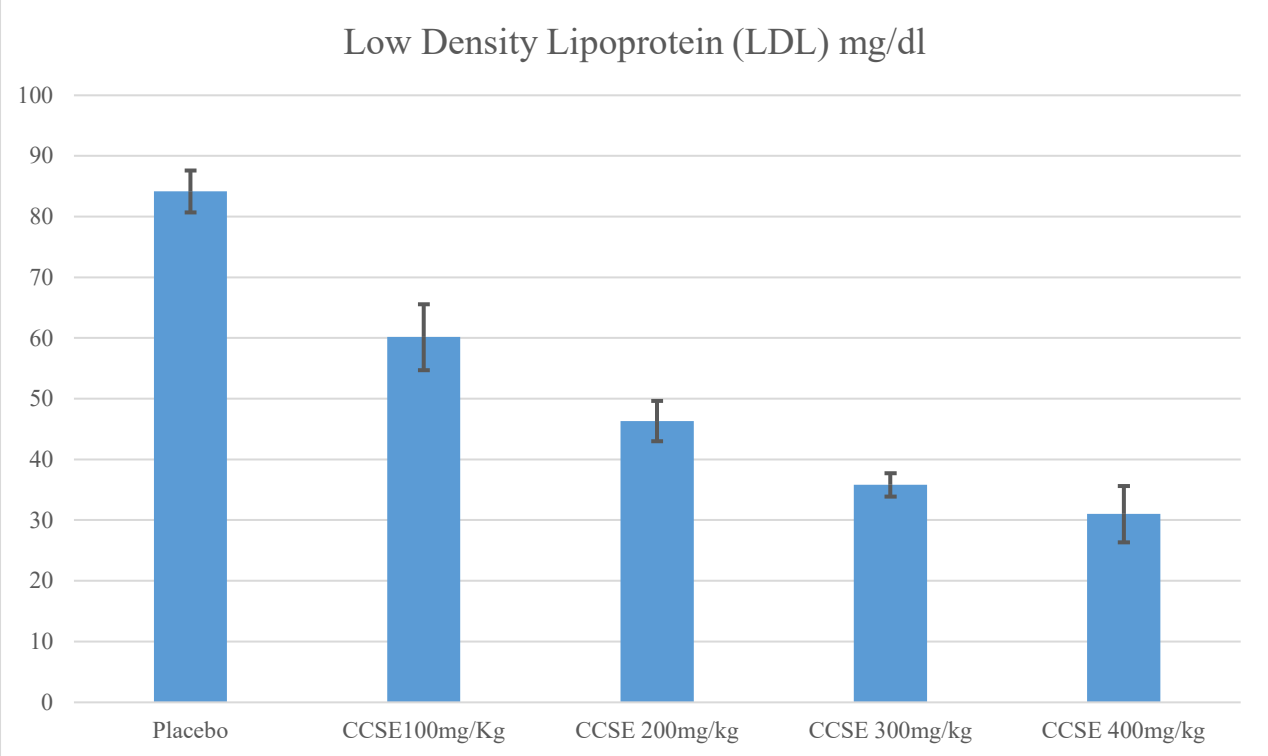


Fig. 3: Functionl expression low density lipoprotein

atherosclerotic cardiovascular diseases. *Citrullus colocynthis* seeds may provide novel therapeutic agents as PCSK9 inhibitors.

Conflict of interest

Authors declared no conflict of interest.

Ethical approval

Ethical approval for handling the animals was obtained from the departmental review and ethical committee of UCCM, Islamia University of Bahawalpur No.904/UCCM.

Table 1: In-silico pharmacokinetics (drug likeliness) studies of *C. colocynthis* compounds

Compound code	Molecular formula	Compound Name	Swiss ADME Analysis						
			Mol. Wt. (g/mol)	LogP (cLogP)	Lipinski's Rule of Five Violation	Skin Permeation Value (log Kp) cm/s	GI Absorption	BBB Permeability	Cytochrome P450 inhibitors
201	C9H18O	Ether, 3-butenyl pentyl	142.24	2.77	0	-5.05	High	Yes	No
202	C8H9NO2	oxime-, methoxy phenyl	151.16	1.69	0	-5.78	High	Yes	No
203	C9H12N2O	4-Dimethylbenzamide	164.2	0.97	0	-7.23	High	Yes	No
204	C7H13NO3	Betain	159.18	-2.19	0	-7.39	High	No	No
205	C6H12ClNO	Ethyl 4-chlorobutanimidoate	149.62	1.81	0	-6.18	High	Yes	No
206	C15H19NO2	Benzofuran, 2,3-dihydro	245.32	2.5	0	-6.27	High	Yes	No
207	C6H18O3Si3	Cyclotrisiloxane, hexamethyl	222.46	1.16	0	-5.51	High	Yes	No
208	C12H36O6Si6	Cyclohexasiloxane, dodecamethy	444.92	1.76	0	-4.73	High	Yes	No
209	C9H10O2	2-Methoxy-4-vinylphenol	150.17	2.14	0	-5.22	High	Yes	Yes
210	C9H2F17NO2	3-Isopropoxy-1,1,1,7,7,7-hexamet	479.09	5.47	0	-5.67	Low	No	No
211	C10H14O	Cymene-7-ol	150.22	2.39	0	-5.55	High	Yes	Yes
212	C10H14O	2,4-Di-tert-butylphenol	150.22	2.39	0	-5.55	High	Yes	Yes
213	C9H21O3P	Triisopropyl phosphite	208.24	2.55	0	-5.82	High	Yes	No
214	C23H26O3	Cyclopropanecarboxylic acid, 2,2	350.45	5.24	1	-4.01	High	Yes	No
215	C19H36O2	1-Octadecenoic acid, methyl est	296.49	5.95	1	-2.82	High	No	Yes
216	C19H36O2	cis-13-Octadecenoic acid, methyl	296.49	5.95	1	-2.82	High	No	Yes
217	C19H38O2	Methyl stearate	298.5	6.24	1	-2.19	High	No	Yes

Table 2: Molecular docking studies of *C. colocynthis* compounds

Compound	Mol. Wt. (g/mol)	Binding energy	H-bond bonding	Alkyl	Pi-alkyl	Pi sigma bond	Unfavorable bond	pi-pi-stacked
202	151.16	-5.9	LEU:436, TRP:461, VAL:435	ARG:458, ILE:416	PRO:438	LEU:436, TRP:461, VAL:435		
203	164.2	-6.5	VAL:359, ARG:357, ALA:649, VAL:435		PRO:438	VAL:359, ARG:357, ALA:649, VAL:435		
206	245.32	-6.5	TRP:461, ALA:328	PRO:331, CYS: 358.Val:460	PRO:331, CYS: 358.Val:460	TRP:461, ALA:328		
209	150.17	-5.9	VAL:359, ARG:357					
211	150.22	-5.6			PRO:438, LEU:440, VAL:650	THR:437	PRO:438, LEU:440, VAL:650	
212	150.22	-5.5	VAL:435	PRO:438				
214	350.45	-7.4		ALA:514	LYS:494, HIS:643, PRO:639		TRP:566	ALA:514

Table 3: Effect of CC extract on PCSK9 mRNA gene expression

Groups	Gene	Tissue	Group-I (HFD-IR) (qRT-PCR)	CCSE treatment group (qRT-PCR)	P-value
Group-II	PCSK9	Hepatic	1.076±0.157	0.662±0.115	<0.046
CCSE 100mg/kg	PCSK9 (18S)	Hepatic	1.038±0.325	0.814±0.252	<0.003
Group-III	PCSK9	Hepatic	1.024±0.462	0.833±0.467	<0.075
CCSE 200mg/kg	PCSK9 (18S)	Hepatic	1.038±0.325	0.893±0.214	<0.2
Group-IV	PCSK9	Hepatic	1.038±0.360	0.331±0.054	<0.001
CCSE 300mg/kg	PCSK9 (18S)	Hepatic	1.038±0.325	0.206±0.044	<0.001
Group-V	PCSK9	Hepatic	1.038±0.325	0.206±0.044	<0.026
CCSE 400mg/kg	PCSK9 (18S)	Hepatic	1.038±0.325	0.277±0.155	<0.014

REFERENCES

Afshari A, Salimi F, Nowrouzi A, Khalili MB, Bakhtiyari S, Hassanzadeh G, Shabani M, Ahadi A and Farhadiannezhad M (2021). Differential expression of gluconeogenic enzymes in early-and late-stage diabetes: the effect of *Citrullus colocynthis* (L.) Schrad. Seed extract on hyperglycemia and hyperlipidemia in Wistar-Albino rats model. *Clin. Phytosci.,* **7**: 1-22.

Ahamad S and Bhat SA (2022). Recent update on the development of PCSK9 inhibitors for hypercholesterolemia treatment. *J. Med. Chem.*, **65**(23): 15513-15539.

Altemimi AB, Mohammed MJ, Alkanan ZT, Abdulrahman SH, Allaitha SA, Abedelmaksoud TG, ALKaisy QH, Najm MA, Cacciola F and Srinivasan PV (2023). Bioactive compounds of *Citrullus colocynthis* aerial parts: characterization and biological properties. *Plant Biosyst.-Bio.*, 157(6) 1-10.

Amin A, Tahir M and Lone KP (2017) Effect of *Citrullus colocynthis* aqueous seed extract on beta cell regeneration and intra-islet vasculature in alloxan induced diabetic male albino rats. *J. Pak. Med. Assoc*., **67**(5): 715-21.

Asif HM and Hashmi HAS (2021). Bioactive Compounds of Ajwain (Trachyspermum ammi [L.] Sprague). In: Murthy, H.N., Paek, K.Y. Editors. Bioactive Compounds in Underutilized Vegetables and Legumes. Reference Series in Phytochemistry. Springer, Cham. Publishing., Switzerland AG., Pp.257-273

Chapa JJ, Jonathan CM, Janina QB, Rishika SP, Maya EG and Roopa AR (2023) PCSK9 inhibition in patients after heart transplantation: A retrospective review and literature analysis. *Curr. Heart Fail. Rep.*, **20** (3): 168-178.

Chen X, Li H, Tian L, Li Q, Luo J and Zhang Y (2020). Analysis of the physicochemical properties of acaricides based on Lipinski's rule of five. *J. Comput. Biol.*, **27**(9): 1397-1406.

Emma MR, Giannitrapani L, Cabibi D, Porcasi R, Pantuso G, Augello G and Cervello M (2020). Hepatic and circulating levels of PCSK9 in morbidly obese patients: Relation with severity of liver steatosis. *BBA-Mol. Cell Biol. Lipids,* **1865**(12): 158792.

He Q, Luo J, Wu J, Yao W, Li Z, Wang H and Xu H (2020). FoxO1 knockdown promotes fatty acid synthesis via modulating SREBP1 activities in the dairy goat mammary epithelial cells. *J. Agric. Food Chem.*, **68**(43): 12067-12078.

Hong, Najiao, Yongjun Lin, Zhirong Ye, Chunbaixue Yang, Yulong Huang, Qi Duan and Sixin Xie (2022). The relationship between dyslipidemia and inflammation among adults in east coast China: A cross-sectional study. *Front. Immunol.*, **13**: 937201.

Iqbal M, Hasanah N, Arianto AD, Aryati WD, Puteri MU and Saputri FC (2023). Brazilin from *Caesalpinia* sappan L. as a Proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitor: Pharmacophore-based virtual screening, *in-silico* molecular docking and *in vitro* studies. *Advance Pharmacol. Pharm. Sci.*, **2023**: 1-10

Li M, Cai Y, Chen X, Zhang L, Jiang Z and Yu Q (2022). Tamoxifen induced hepatic steatosis in high-fat feeding rats through SIRT1-Foxo1 suppression and LXRSREBP1c activation. *Toxicol. Res.,* **11**(4): 673-682.

Li QY, Munawar M, Saeed M, Shen JQ, Khan MS, Noreen S, Alagawany M, Naveed M, Madni A, and Li CX (2022). *Citrullus colocynthis*(L.) Schrad (Bitter Apple Fruit): Promising traditional uses, pharmacological effects, aspects and potential applications. *Front. Pharmacol.*, **12**: 791049.

Lin YK, Yeh CT, Kuo KT, Yadav VK, Fong IH, Kounis NG, Hu P, and Hung MY (2021) Pterostilbene increases LDL metabolism in HL-1 cardiomyocytes by modulating the PCSK9/HNF1α/SREBP2/LDLR signaling cascade, upregulating epigenetic hsa-miR-335 and hsa-miR-6825 and LDL receptor expression." *Antioxidants*, **10**(8): 1280.

Liu H, Lin C, Wang S, Xiao S, Cheng X, Zheng M, Chen S, Zeng X, and Chen S. (2022). A SYBR green I-based qRT-PCR Assay for detecting Duck BMPs. *Fujian J. Agric. Sci.*, **37**(12): 1509-1518.

Mahmoudi A, Butler AE, Banach M, Jamialahmadi T and Sahebkar A (2023). Identification of potent small-molecule PCSK9 inhibitors based on quantitative structure-activity relationship, pharmacophore modeling and molecular docking procedure. *Curr. Probl. Cardiol.*, 101660.

Manzoor A, Khan IA, Kousar S, Iqbal MO, Munawar SH, Manzoor Z, and Perwasha P. (2022) Evaluation of cardioprotective potential of hydroalcohol peel extract of *Citrullus colocynthis* Linn.(Cucurbitaceae). *Trop. J. Pharm. Res.*, **21**(1): 105-110.

Päth G, Perakakis N, Mantzoros CS, and Seufert J. (2022) PCSK9 inhibition and cholesterol homeostasis in insulin producing β-cells. *Lipids health Dis.*, **21**(1): 138.

Rajpoot SR, Ahmad K, Asif HM, Madni MA, Tasleem MW, Zafar F, Majeed H, Khalid A, Hashmi HAS and Aslam MR (2024). Study of anti-inflammatory and anti-arthritic potential of curcumin-loaded Eudragit L100 and hydroxy propyl methyl cellulose (HPMC) microparticles. *Polym. Bull.*, **81(5)**: 4335-4350.

Sindhu HA, Afzal M, and Shahid I (2023) Pharmacological activities and in-silico studies of bioactive compounds identified in organic fractions of the methanolic extract of *Citrullus colocynthis*. *Dose-Response*, **21**(3): 1-15.

Thamer FH and Thamer N (2023) Gas chromatography-Mass spectrometry (GC-MS) profiling reveals newly described bioactive compounds in *Citrullus colocynthis* (L.) seeds oil extracts. *Heliyon*, **9**(6): 1-9.

Youl EN, Nassouri S, Ilboudo S, OuÃ M, SombiÃ CB, Ilboudo S and Guissou IP (2020). Hypoglycemic and antihyperglycemic activities of the aqueous ethanolic extracts of *Gymnema sylvestre* (RETZ) R. Br. Ex

SCHULT and *Sclerocarya birrea* (A RICH) HOCHST. *Afr. J. Pharm. Pharmacol.*, **14**(9): 339-346.

Zainab R, Kaleem A, Ponczek MB, Abdullah R, Iqtedar M, and Hoessli DC (2021). Finding inhibitors for PCSK9 using computational methods. *PLoS One*, **16**(8): e0255523.

Zhang Y, Ezrokhi M, Cominos N and Cincotta AH (2020). 1823-P: Bromocriptine (BC) normalizes elevated expression of liver genes involved in fatty acid oxidation, lipogenesis, inflammation and endoplasmic reticulum (ER) stress and glucose intolerance of high-fat/sucrose diet-fed rats. *Diabetes,* **69**(Supp_1): 1823.