Original Article

Bioinformatics and Chemoinformatics Analysis Explored the Role of *Linum usitatissimum* in Diabetic Heart Conditions: Experimental Analysis in H9c2 Rat Embryonic Cardiomyocytes Cell Lines

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Abstract

Background: Cytokine storms and inflammation lead to heart failure (HF). Bioactive compounds, as complementary medicine, can be the primary source of compounds with anti-inflammatory properties. *Linum usitatissimum* (LiU) has antioxidant capacity and anti-inflammatory activity. Here, candidate hugeness was selected based on the *in silico* studies, bio-cheminformatics, and bioinformatic analysis for excremental validation. **Methods:** We selected the vital genes with differential expression from the GSE26887 dataset. Based on the bioinformatics analysis, several parameters are determined to choose switchable genes involved in diabetic HF (DHF). We designed the protein–protein interactions network to consider the nodes' degree, modularity, and betweenness centrality. Hence, we selected the interleukin (IL)-6 protein as a target for drug design and discovery to reduce diabetes complications in the heart. Here, H9c2 cell lines of rat embryonic cardiomyocytes induce HF using hyperglycemic and hyperlipidemic conditions. Real-time polymerase chain reaction evaluated the relative expression of SMAD7/NRF-2/STAT3. Furthermore, we assessed the concentration of IL-6 using the enzyme-linked immunosorbent assay technique. **Results:** Based on the bioinformatic analysis, we found that IL-6 with the highest network parameters score might be presented as a druggable protein in the DHF. Bioactive compounds and phytochemicals have potential strategies to manage DHF. LiUs decreased the expression level of the SMAD7 (P <0.0001) and STAT3 (P < 0.0001), and increased the expression level of the NRF2 (P < 0.0001). In addition, LiUs significantly reduced the concentration of IL-6 (P < 0.0001). **Conclusion:** Our data proposed that LiUs regulated inflammation and triggered the antioxidant defense in HF. Moreover, LiUs could have potential approaches to managing and preventing DHF.

Keywords: *Bioinformatics and chemoinformatic, complementary medicine, diabetic heart failure, H9c2, inflammation, Linum usitatissimum*

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Introduction

The most frequent cause of death is cardiovascular disease (CVD), which is caused by metabolic abnormalities in patients with type 2 diabetes mellitus (T2DM).[1,2] To reduce CVD morbidity and mortality, it is essential to manage patients with Type 2 diabetes (T2D) by controlling blood glucose levels and enhancing cardiometabolic parameters.[3]

High blood sugar levels are the hallmark of diabetes, an endocrine condition. The statistical estimation will predict approximately 693 million people with diabetes status worldwide by 2045.[4,5] Vascular complications, which affect both the macrovascular and microvascular systems, account for the vast majority of all diabetes-related hospitalizations and deaths, placing a substantial financial burden on both developed and developing societies.[4,5] In addition, evidence has indicated that there is an association between atherosclerotic CVD and diabetes as one of the diabetic complications.[6]

Chronic inflammation has a role in various diseases and mortality.[7] Growing studies have indicated that the tumor necrosis factor-alpha (TNF-α), interleukin (IL)-6, JAK, and STAT signaling pathways are associated with inflammation and oxidative stress in the function of cardiac tissue.[8] The inflammatory cascade might induce the response to external and endogenous factors

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such as tissue damage, traumas, and disease.[9,10] Moreover, several signaling pathways could modulate heart function, leading to the progression of new therapeutic strategies.[11-14]

Oxidative stress and inflammation have been related to the pathogenesis of heart failure (HF), which dysregulated cardiac tissue pathophysiology.[11-14] Immense studies indicated that hyperglycemia and hyperlipidemia might trigger oxidative stress and inflammation. Hence, increasing the inflammatory agents and reactive oxygen species led to myocardial dysfunction.[15] At the molecular level, the significant differential expression profile changes homeostasis and intracellular adjusting.[16] Several signaling pathways and protein dysfunctions have been identified in HF etiology.[15] As a vital target in oxidative heart stress and inflammation processes, the IL-6/SMAD7/STAT3/NRF-2 pathway has been recognized as an essential gene axis in the molecular mechanism of diabetic HF (DHF). When conventional pharmaceuticals are used with complementary medicine, positive health outcomes may result from treatment.[17]

Dietary supplements have the potential to regulate blood glucose and other cardiometabolic parameters. Dietary supplements have a promising track record of regulating blood glucose and other cardiometabolic parameters.[18] Due to its abundance of alpha-linolenic acid (ALA), lignans, dietary fiber, and other substances that may be beneficial to health, *Linum usitatissimum* (LiUs), which is considered a functional food, has become popular.[19]

Phytochemicals can be found in flaxseed, which has been shown to have potential health benefits in several studies.[20] Previous reports indicate that flaxseed oil and its fractions possess significant medicinal properties, including antioxidant, anti-inflammatory, anticancer, antiviral, and bactericidal properties. Moreover, they can reduce blood glucose and cholesterol levels.[21] Phytochemicals extracted from flaxseeds have primarily focused on the bound lignan macromolecule complex, while the unbound polar fraction has been neglected significantly.[22] LiUs oil is a type of herb that has been detected to have substantial beneficial effects, mainly the decrease of blood lipids,[22] hepatoprotective effects, prevention of many CVDs, and anti-diabetic characteristics. LiUs have the highest amount of lin plant foods, approximately 800 times more than other plant foods consumed.[23]

LiUs may effectively treat various illnesses and diseases.[24] The majority of studies revealed that LiUs and LiUs extract could attenuate hyperglycemia in T2D and improve HF symptoms. However, several investigations suggested that there could be harmful chemicals in LiUs. Consuming a moderate amount of LiUs has been proven to have minimal adverse effects.[25-29]

In this study, we apply chemobioinformatics and molecular modeling to search for the appropriate bioactive chemicals in LiUs. In addition, we used *in silico* analysis to identify the master hub genes responsible for inflammation cascade and oxidative stress state DHF condition monitoring. Therefore, the primary purpose of this research is to examine the impact of lignans, linolenic acid, and secoisolariciresinol diglucoside (SDG) as significant components of LiUs on inflammation cascade and oxidative stress stat in adherent H9c2 cell lines.

Methods

System biology analysis and molecular docking approach

The Research Ethics Committees of the Islamic Azad University – Isfahan (Khorasgan) Branch agreed on the research project in all of its protocols. The current research applied network visualization tools to examine gene expression data, leading to the generation of a model emphasizing the course of DHF. The study identified biochemical components present in LiUs that have the potential to impact patients' life expectancy and survival rates. The study of pathogenic mechanisms implicated in the progression of DHF includes the analysis of the protein–protein interaction (PPI) network and the fundamental molecular signaling pathways that are pivotal in the pathogenesis of this condition.

Analyzing raw data of microarray in the diabetic heart failure condition

In the current study, exploring NCBI and GEO databases and analyzing the GSE26887 dataset, we achieved a significant differential expression profile of the genes involved in DHF, which was analyzed using the R programming language software and Bioconductor packages. Data normalization was supported using the RMA method. A comparison analysis was conducted on the datasets using a *t*-test to identify genes with significant differential expression. A predetermined significance level of $P < 0.05$ was used. The heatmap diagram was constructed using the ggplot program, identifying genes with $P < 0.001$.

On the other hand, we used a log fold change (logFC ± 0.09) threshold to group together the overexpressed and downregulated genes, thereby specifying the genes that have significant significance. In the second step, the STRING 11.5 database created a network of PPIs consisting of hub nodes obtained from each dataset analysis. The network was constructed using a medium confidence level.

Based on the Cytoscape algorithms and applying major network parameters such as degree: 10, betweenness centrality: 0.01, ad closeness centrality: 0.15, on the PPIs network of significant differential gene expression with $P < 0.05$ and logFC ± 0.09, we distinguished that hub genes contribute to HF in diabetes status. Here, we

designed a genetics interaction network of hub genes as a master switching network in the DHF hallmarks occurrence by Gephi software. Furthermore, the hub genes list was enriched in the Reactome, KEGG, and Enrichr to highlight significant molecular signaling pathways associated with diabetic complications in the heart tissue.

Molecular docking virtual screening

This research analyzed a druggable target with a negative linear dependency on survival rates to identify possible synergistic effects in pharmacological interventions. Potential targets for drug development and antagonist selection based on drug design principles were identified as the genes with the greatest betweenness centrality. According to betweenness centrality, degree, and eigenvector scores, IL-6 is defined as a master switching and druggable protein in this genetic interaction network. Hence, we selected the IL-6 protein as a target for drug design and discovery to reduce diabetes complications in the heart. The X-ray diffraction structure of the protein IL6 (protein data bank [PDB] ID: 4NI7) was obtained and analyzed using the PDB repository.[30] This structure was experimentally determined and rendered in a three-dimensional (3D) format. The literature study yielded the identification of the most bioactive components. The process of retrieving and preserving active chemicals in a 3D structural format (SDF) from the PubChem server[31] and then incorporating them into the chemical library was carried out using the Open Babel software.[32] The preparation and optimization of the 3D structure of IL-6 were undertaken before evaluating the binding affinity between the macromolecule IL-6 and the chemical library. We used the Dock Prep tools in UCSF Chimera 1.8.1 to remove extra chains, noncomplex chemicals, ligands, ions, and solvents.[33] The molecular docking estimate was performed using the PyRx virtual screening platform[34] after undergoing energy reduction and translating the chemical library from SDF format to PDB-QT format. Vina search space in PyRx software was applied in the dimensions x: 49.0486 Å, y: 45.8160 Å, and z: 55.7787 Å. The ideal treatment target was selected based on two criteria: a binding affinity <-5 and a root mean square deviation (RMSD) <2, as established by molecular docking research. We used PyMOL[35] and BIOVIA Discovery Studio Visualizer software version 2021 to discover the interaction between medicinal chemistry components of phytochemicals cocktail and macromolecules (IL-6).

Preparation of *Linum usitatissimum* and oil extraction

Brown LiUs (*Linum usitatissimum L.*) were purchased from the medicinal plants center in Isfahan, Iran. After being rinsed, the LiU seeds were powdered in an electric mixer at room temperature. Then, 500 mL of hexane for 50 g of powdered LiUs was used for extraction. After 12 h of evaporating the powdered meal at room temperature in the hood, 200 mL of 100% methanol, 100% ethanol, and 80% ethanol were extracted. First, the recovered supernatant solvent was filtered using Whatman No. 1 Filter Paper to remove the large particles. Next, a rotary evaporator concentrated the extracts to dry at 45°C in a vacuum. Finally, before dissolving the extracts in a tiny amount of the original solvent and keeping them at −20°C, we weighed them to calculate the yield.

Cell culture protocol

We purchased an adherent H9c2 rat embryonic cardiomyocyte cell line from the Pasteur Institute of Iran in this study. We defined three groups, including (1) normal group; (2) DHF induced by hyperglycemic and hyperlipidemic conditions; and (3) DHF + LiUs bioactive compounds (DHF + LiUs). Cells were maintained in a sterile environment by cultivating them in Dulbecco's Modified Eagle Medium (DMEM) (Capricorn Scientific DMEM-LPXA) with 10% fetal bovine serum (FBS) (Capricorn Scientific FBS-11A) at 37°C and 5% carbon dioxide. In addition, penicillin and streptomycin (100 U/mL and 100 g/mL) were added to cell culture media to avoid contamination. There were 1×10^6 cells/mL in each 6-well cell culture plate. At 70% confluence, cells from 10 to 12 passages were sampled from several vials. Normal cells were cultivated in DMEM containing 5.5 mM of glucose. In contrast, hyperglycemic cells were maintained in a medium containing 50 mM glucose (G 25 mM and G 33 mM). Moreover, 1% bovine serum albumin and 500 M palmitic acid (PA, PO500 Sigma-Aldrich) were used to create a hyperlipidemic medium (BSA, A3675 Sigma-Aldrich). An appropriate 5 mM PA/10% BSA stock solution may be prepared by combining 0.5 mL of a 100 mM PA solution in 0.1 M NaOH with 9.5 mL of a 10.5 m/v BSA solution in distilled water. Hyperglycemic (25 mM or 33 mM glucose) and hyperlipidemic (25 mM or 33 mM glucose) media were prepared by adding 1/10 of the stock solution five mM PA/10% BSA to a final concentration of 500 M/1% BSA. In the 1% BSA control group, we saw no significant differences from the norm. The H9c2 cell line was treated with 150 M LiUs extract dissolved in dimethyl sulfoxide (Merck, Whitehouse Station, NJ, USA) for 24 h.

Enzyme-linked immunosorbent assay

To measure the level of IL-6 in the cell culture after treatment with LiUs, we centrifuge the cell culture medium at a speed of 2000 g for 10 min to remove the supernatant. Then, the supernatants were collected, and the experiment was conducted based on manufacturer company protocol. Samples were diluted using Sample Diluent NS, and undiluted samples were kept at a temperature of −20°C or below. In this protocol, we should refrain from frequent freeze–thaw cycles.

Relative expression assay

TRIzol was used to isolate total RNA from the cell line using RNase-free procedures (Thermo Scientific, USA). We employed the DNase I endonuclease according to the

DNase I Treatment Kit (TaKaRa, Japan) methodology to boost the purity of extracted total RNA samples and eliminate DNA. cDNAs were also generated using reverse transcriptase enzyme (TaKaRa, Kusatsu, Shiga Prefecture, Japan). We used SYBR Green dye (TaKaRa, Kusatsu, Japan) and quantitative real-time polymerase chain reaction (PCR) (Rota-Gene 6000 apparatus, Corbett Life Science) to determine the relative expression of genes [Table 1]. The timeline of real-time PCR is presented in Table 2. As a reference gene, glyceraldehyde-3-phosphate dehydrogenase was utilized to determine the normalization of gene expression data. In addition, this work calculated the fold-change analysis of mRNA expression using the $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

We calculated the statistical analysis by GraphPad Prism Software (Version 9, GraphPad Software Inc., La Jolla, CA, USA). Furthermore, the results were assessed using a one-way analysis of variance followed by Tukey's *post hoc* test to control for multiple comparisons. In this study, statistical significance was analyzed at $P \leq 0.01$. Moreover, data are presented using the mean and standard deviation.

Results

System biology results

According to the GSE26887 dataset analysis, 2844 genes in the DHF biopsies had significant differential expressions compared to healthy biopsies ($P < 0.05$). Genes with significant differential expression ($P < 0.001$) are visualized in the heatmap diagram [Figure 1a]. Notably, we documented 554 downregulated and 873 genes overexpressed in diabetic heart status. Moreover, in enforcing network parameters, we achieved 47 nodes with the highest degree and betweenness centrality as hub genes related to diabetic heart incidence. Moreover, the genetic interaction network of 47 hub genes marked IL-6, SMAD7, STAT3, and NRF-2 as master nodes with the highest betweenness centrality [Figure 1b]. Enriching 47 hub genes revealed the significant molecular signaling pathways and biological processes associated with hub genes [Figure 2]. Based on enrichment data, regulation of metabolism and gene expressions, HIF1 signaling pathway, cellular response to cytokine stimulus, PI3K-Akt signaling pathway, regulation of gene expression, Inflammatory response, positive regulation of IL-6 production, cytokine signaling in immune system, signaling by receptor tyrosine kinase, SMAD2/SMAD3/SMAD4 heterotrimer regulate transcription, STAT3 nuclear event downstream, and myocardial infraction are significant molecular signaling pathways and biological processes associated with hub genes; Hence, proposed these molecular signaling pathways and biological processes have interdependence with DHF pathogenesis.

Molecular modeling outputs

Based on bioinformatic analysis, we found that IL-6, a significant cut-point protein in the DHF network and as a druggable node, could affect diabetes complications in the cardiomyocytes. Based on our findings, the molecular docking method estimated the binding affinity between bioactive compounds of LiUs extract on the IL-6 macromolecule. The molecular docking analysis outcomes indicated the catalog of the binding affinity of bioactive components derived from flaxseed, which targets IL-6 macromolecules. The highest binding affinity belongs to lignan and SDG bioactive compounds, respectively (binding affinity = −7.2 kcal/mol, RMSD <2 and binding affinity = −6.1 kcal/mol, RMSD <2) [Figure 3a-c and Table 3]. Moreover, PyMol and BIOVIA Discovery Studio Visualizer software discover the interaction between medicinal chemistry components of phytochemicals cocktail and IL-6 [Figure 3d-f].

Linum usitatissimum improved regulation of the interleukin-6/SMAD7/STAT3/NRF-2 network

The concentration of IL-6 enhanced in the DHF group [$P < 0.0001$, Figure 4a]. Furthermore, we found that the relative expression of the SMAD7/STAT3 axis significantly increased in the DHF group [$P < 0.0001$, Figure 4b and c]. On the other hand, the relative expression level of the NRF-2 decreased in the H9c2 cell line [$P < 0.0001$, Figure 4d]. In addition, this study determined that LiUs have anti-inflammatory and antioxidant properties. The data indicated that the concentration of IL-6 declined in the DHF + LiUs group [$P < 0.0001$, Figure 4a]. Moreover, in treatment with LiUs, the relative expression of SMAD7 and STAT3 was reduced in the DHF group [$P < 0.0001$,

Table 1: Primer sequences

Gene symbol	Primer sequence	Annealing temperature	Product size
STAT3	Forward: ACCCAACAGCCGCCGTAG	60	192
	Reverse: CAGACTGGTTGTTTCCATTCAGAT		
SMAD7	Forward: GGCTGTGTTGCTGTGAATC	58	118
	Reverse: GGTATCTGGGAGTAAGGAGGAG		
NRF2	Forward: GCCATTAGTCAGTCGCTCTC	58	98
	Reverse: GTGCCTTCAGTGTGCTTCT		
GAPDH	Forward: TTCAACAGCAACTCCCATTC	60	102
	Reverse: GCCATATTCATTGTCATACCAG		

GAPDH – Glyceraldehyde-3-phosphate dehydrogenase

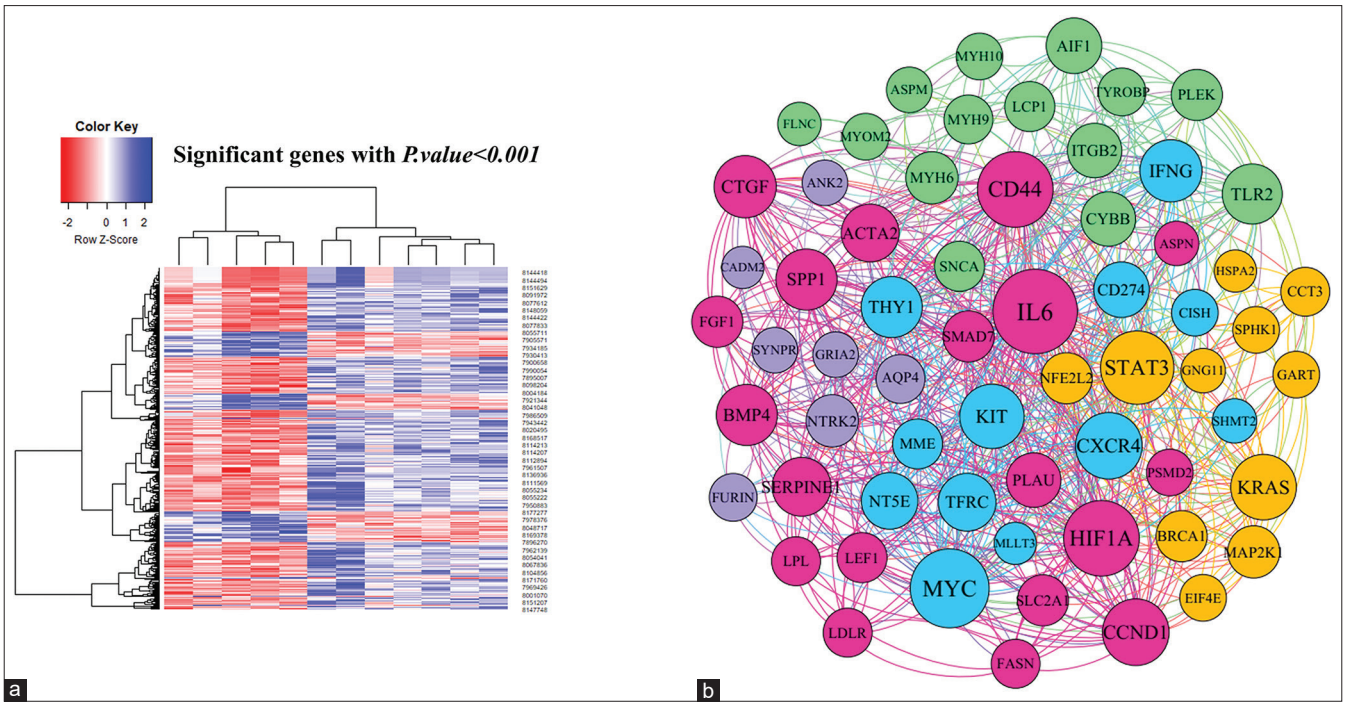

Figure 1: Schematic of system biology in diabetic heart conditions. (a) The heatmap diagram showed the significant differential expression of genes in the diabetic heart compared to normal samples with *P* < 0.001 based on R programming analysis and Bioconductor and oligo packages. (b) The protein–protein interactions network of hub genes in diabetic heart conditions by considering network parameters (degree, modularity, and betweenness centrality) in the Gephi platform

Table 2: Real-time polymerase chain reaction timeline

	Stage						
	Stage 1		Stage 2	Stage 3			
Temperature (°C)	95	95	Annealing temperature (°C)	72	95	60	95
Time (s)	30	5	10	30	15	60	15
Repetition	×1		×45			×1	

Figure 4b and c]. Interestingly, in the DHF + LiUs group, the expression level of NRF-2 was remarkably higher than in the DHF condition group [$P < 0.0001$, Figure 4c].

Discussion

In silico analysis indicated that the IL-6/STAT3/SMAD7/NRF2 network could regulate the signaling pathway in DHF conditions. In addition, the computational system biology analysis indicated that IL-6 and STAT3 could be vital molecules in the DHF condition. Furthermore, the enrichment of hub genes indicated the major molecular signaling pathways implicated in DHF, including TNF-α signaling, inflammatory response, IL-6/JAK/STAT signaling, PI3K/Akt, Hif-1α, and apoptosis. Moreover, we investigated the anti-inflammatory and antioxidant capacity of LiUs bioactive compounds as a new perspective on complementary and alternative medicine strategies in an *in vitro* model of adherent H9c2 cell line of rat embryonic cardiomyocytes exposed to low-grade inflammation. In this study, the expression of the SMAD7/STAT3 was significantly enhanced in the DHF group induced by hyperglycemic and hyperlipidemic conditions. Moreover, the concentration of IL-6 decreased in the DHF group induced by hyperglycemic and hyperlipidemic conditions.

Evidence indicated that consuming 8 g/kg BW of crude LiUs for 12 weeks reduced inflammation, lipid indices, and atherosclerotic indicators.[36] A comprehensive and meta-analysis research revealed that LiU supplementation might reduce circulating c-reactive protein (CRP), VCAM-1, and IL-6 in adults.[37] To evaluate the anti-inflammatory potential of phytochemicals, an adherent H9c2 cell line of rat embryonic cardiomyocytes was employed to imitate the inflammatory and oxidative milieu observed in clinically important chronic inflammatory diseases. Based on *in vitro* modeling, we exposed H9c2 cardiac cells to hyperglycemic and hyperlipidemic conditions to assess the effect of LiUs on inflammation, antioxidant status, function, and myogenesis.

The use of supplements to improve glycemic control in the therapeutic of T2DM and prediabetes is currently not supported by sufficient evidence.[38] The recommendation suggests consuming foods rich in dietary fiber to achieve the recommended fiber intake, as they contain micronutrients and phytochemicals. To achieve optimal nutrition, it is recommended to eat foods that are rich in omega-3 fatty acids, such as fish, nuts, and seeds, including LiUs.[19]

The *Linaceae* family has a herbaceous plant called LiUs that produces flaxseed. Due to its attractive nutritional

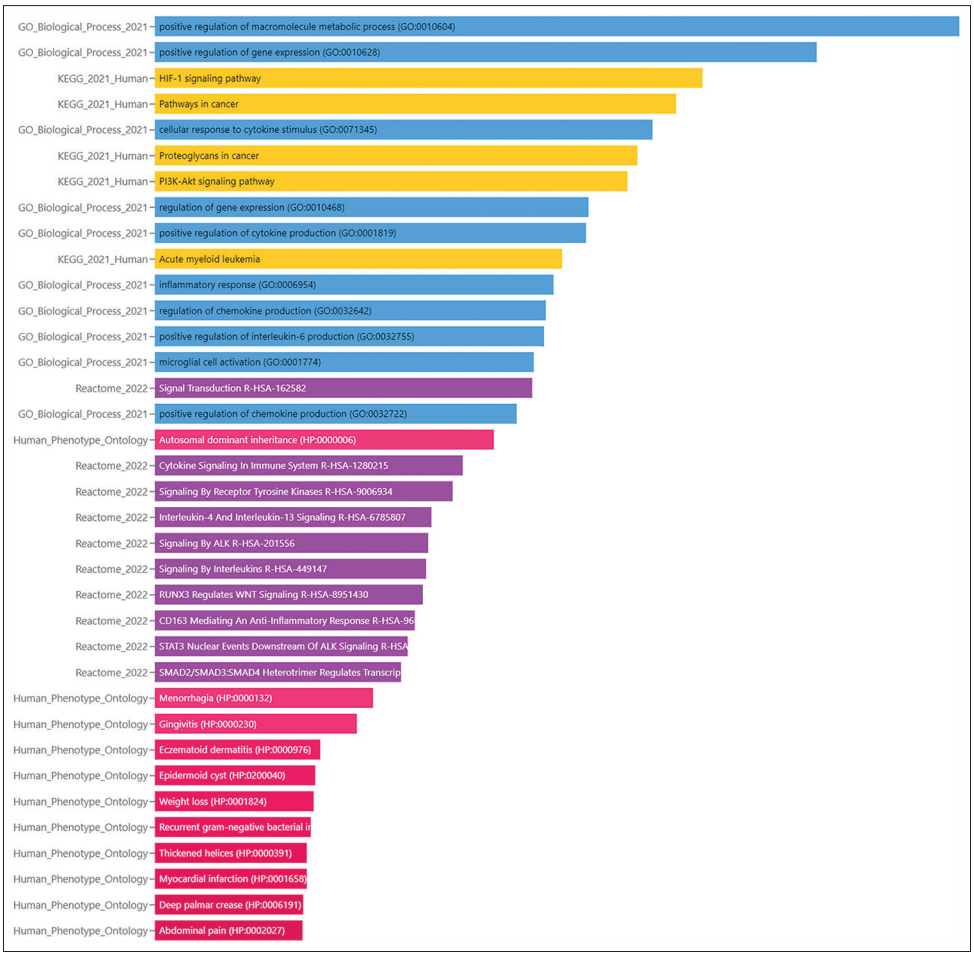

Figure 2: Enrichment of hub genes based on molecular and cellular processes and signaling pathways. Regulation of metabolism and gene expressions, HIF1 signaling pathway, cellular response to cytokine stimulus, PI3K-Akt signaling pathway, regulation of gene expression, Inflammatory response, positive regulation of interleukin-6 production, cytokine signaling in the immune system, signaling by receptor tyrosine kinase, SMAD2/SMAD3/SMAD4 heterotrimer regulate transcription, STAT3 nuclear event downstream, and myocardial infraction are significant molecular signaling pathways and biological processes associated with hub genes based on Enrichr-KG platform

content, it is considered a functional food. It is the most significant source of ALA along with its high dietary fiber content, proteins, and phytoestrogens.[18]

LiU supplementation can have beneficial effects on lipid profiles, anthropometric measures, and inflammatory cytokines such as IL-6 and CRP.[37,39] Whole LiUs were shown by a meta-analysis to be the most effective way to improve glycemic control and insulin resistance, but this effect was not exclusively assessed in patients with T2D and prediabetes. Furthermore, the phytochemicals found in LiUs have attracted a lot of attention as bioactive molecules that are beneficial for health.[37,40] Potential health benefits of LiU oil, fibers, and flax lignans include the ability to reduce atherosclerosis, arthritis, diabetes, CVD, osteoporosis, cancer, and neurological and autoimmune disorders. Noreen and Kaur in the separate studies have indicated that therapeutic effect of LiUs on diabetic nephropathy.[41,42] Kaur *et al*. have shown that administration of LiUs might suppress oxidative stress signaling pathways and improve renal parameters, level of antioxidant enzymes, lipid profile, and glycemic status in diabetic nephropathy.[42] Prasad demonstrated that LiUs could reduce low-density lipoprotein cholesterol, triglyceride levels, total cholesterol, and augmented high-density lipoprotein cholesterol levels.[43]

Combining complementary medicine with commercial/conventional pharmaceutical medication may impact the treatment of cardiovascular disorders. Herbal medicine is one of the most commonly utilized forms of complementary and alternative medicine.[17] Growing evidence has demonstrated that LiUs are a healthful food with antitumor, anti-inflammatory, antithrombotic, antidiabetic, and cardioprotective properties.[24,44-46] LiU, as an oilseed, contains several bioactive compounds, including lignans, linolenic acid, linoleic acid, essential amino acids, cyclopeptide, cyanogenic glycosides, alkaloids, and poly-carbohydrates, according to the results of prior chemical studies. In light of this, we found that lignan and SDG are effective bioactive compounds based on targeting IL-6, a druggable cut-point protein. Furthermore, Ranchoux *et al*. recently reported that IL-6/STAT3 was

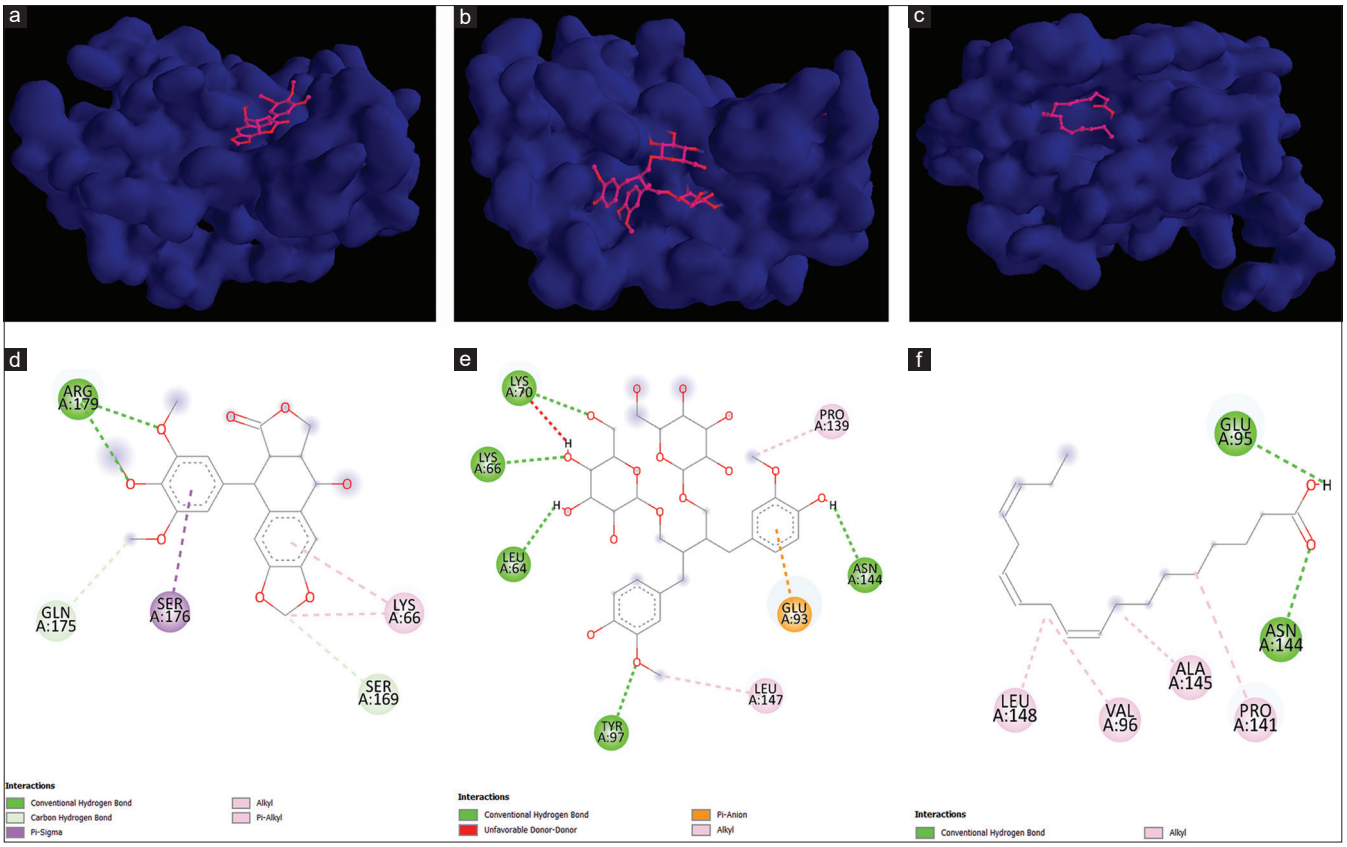

Figure 3: Molecular docking in targeting therapy of interleukin (IL)-6 protein and diabetic heart protein–protein interactions network. (a) Screening of molecular docking showed that small lignan molecule to the active site of IL-6 protein with binding affinity score −7.2 kcal/mol and root mean square deviation (RMSD) <2. (b) As an effective bioactive compound based on targeting IL-6, SDG binds to IL-6's active site with a binding affinity score of −6.1 kcal/mol and RMSD <2. (c) Screening of molecular docking showed that linolenic acid has not an acceptable binding affinity score (−4.5 kcal/mol) and RMSD <2 in the PyRx software and Vina search space in the dimensions x: 49.0486 Å, y: 45.8160 Å, and z: 55.7787 Å. (d-f) PyMol and BIOVIA Discovery Studio Visualizer software discover the interaction between medicinal chemistry components of phytochemicals cocktail and IL6

the significant target for switching signaling pathways for metabolic syndromes and diabetic complications in the cardiac.[47]

Our data indicated that the relative expression of the NRF2 was significantly decreased in the DHF group induced by hyperglycemic and hyperlipidemic conditions. Notably, we found that LiUs improved the regulation of NRF-2. Furthermore, Gao *et al*. revealed that consuming the LiUs bioactive compounds enhanced the expression level of the NRF-2.[48] In addition, Shi *et al*. demonstrated that the α-lipoic acid could improve lipid peroxidation through regulating the NRF-2 expression.[49]

This study has some limitations, such as not measuring the protein level of genes or considering the regulated agent (microRNAs and long noncoding RNA). Moreover, for future studies, we suggested that LiU phytochemicals are implicated in preclinical and clinical studies. Researchers might utilize these findings to direct future studies into developing LiU-based anti-inflammatory and antioxidant medicines.

Conclusion

Experimental findings indicated that LiU phytochemicals might prevent, treat, and manage inflammation and related illnesses. Furthermore, we recommended that LiU phytochemicals could be considered as treatment strategies for clinical practice or dietary recommendations. Our experimental findings suggested that the *Linum usitatissimum* compounds might enhance the function and myogenesis of H9c2 cardiac cells under hyperlipidemic and hyperglycemic conditions.

Declarations statement

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

Availability of data and materials

The data and materials supporting this study's findings are available from the corresponding author on reasonable request.

Table 3: Molecular docking outcomes

Ligand	Binding affinity	RMSD/ub	RMSD/lb
IL6_modified_9917980_uff_E=686.60	−6.1	0	0
IL6_modified_9917980_uff_E=686.60	−6	5.714	1.531
IL6_modified_9917980_uff_E=686.60	−6	2.092	1.443
IL6_modified_9917980_uff_E=686.60	−5.9	17.741	12.925
IL6_modified_9917980_uff_E=686.60	−5.8	18.886	13.033
IL6_modified_9917980_uff_E=686.60	−5.7	17.794	12.944
IL6_modified_9917980_uff_E=686.60	−5.7	19.556	13.288
IL6_modified_9917980_uff_E=686.60	−5.7	18.666	12.999
IL6_modified_9917980_uff_E=686.60	−5.5	17.762	12.5
IL6_modified_5280934_uff_E=142.21	−4.5	0	0
IL6_modified_5280934_uff_E=142.21	−4.4	5.502	2.701
IL6_modified_5280934_uff_E=142.21	−4.4	2.665	1.658
IL6_modified_5280934_uff_E=142.21	−4.4	3.032	1.383
IL6_modified_5280934_uff_E=142.21	−4.4	4.686	1.696
IL6_modified_5280934_uff_E=142.21	−4.3	2.996	1.916
IL6_modified_5280934_uff_E=142.21	−4.3	2.667	1.709
IL6_modified_5280934_uff_E=142.21	−4.2	6.115	3.992
IL6_modified_5280934_uff_E=142.21	−4.2	4.125	1.696
IL6_modified_443013_uff_E=788.01	−7.2	0	0
IL6_modified_443013_uff_E=788.01	−7	2.326	0.652
IL6_modified_443013_uff_E=788.01	−6.8	28.679	24.7
IL6_modified_443013_uff_E=788.01	−6.7	26.722	23.492
IL6_modified_443013_uff_E=788.01	−6.7	28.895	24.74
IL6_modified_443013_uff_E=788.01	−6.5	27.724	23.99
IL6_modified_443013_uff_E=788.01	−6.4	26.481	23.598
IL6_modified_443013_uff_E=788.01	−6.3	26.85	23.156
IL6_modified_443013_uff_E=788.01	−6.3	20.315	15.432

RMSD – Root mean square deviation

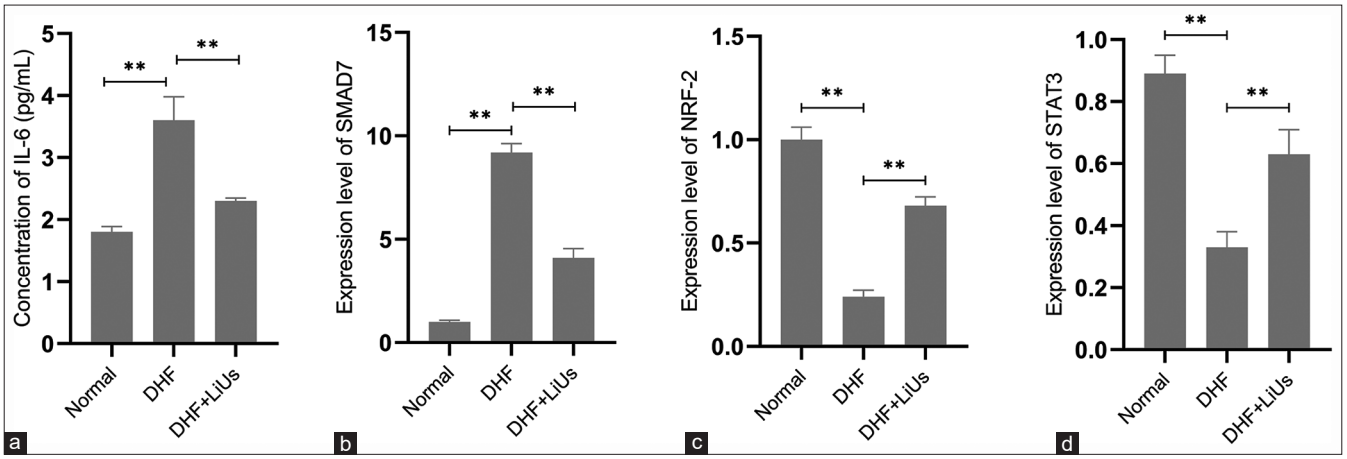

Figure 4: **Indicates the significant between groups (*P*<.05). *Linum usitatissimum* (LiU) treatment modulated the concentration of interleukin (IL)-6 and expression level of the SMAD7/STAT3/NRF-2. (a) IL-6 (pg/mL) concentration was measured by the ELISA method. (b-d) The expression profile of the SMAD7, STAT3, and NRF-2. DC: Diabetic cardiomyocyte condition, DC + LiUs: Diabetic cardiomyocyte + *Linum usitatissimum*. LiUs: *Linum usitatissimum*, IL: Interleukin, DHF: Diabetic heart failure

Author contributions

The study was planned with PN, NA, and FHB. The study was conducted with PN, FHB, and KS. Moreover, NA and FHB approved the data collection, NA, KS, and FT. Interpretation of data was carried out by FHB and NA. The manuscript was drafted with PN and NA. All authors have approved the final draft and agreed to submit the manuscript to this journal.

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Conflicts of interest

There are no conflicts of interest.

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