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Anti-Inflammatory Effects of Eicosapentaenoic Acid and Docosahexaenoic Acid Derived from Patin Fish Oil on Diabetic Nephropathy: A Bioinformatics Study

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Abstract: Diabetic nephropathy is a severe complication of diabetes mellitus with a significant global impact on end-stage renal disease. Fish-derived fatty acids show promise in inflammatory disorders, but their mechanisms in diabetic nephropathy remain unclear. This study used network pharmacology and molecular docking to investigate the therapeutic targets of EPA and DHA from Patin fish oils. Potential targets of EPA and DHA were retrieved from the Swiss Target Prediction, SEA, and SuperPRED databases, identifying 160 and 185 targets, respectively. Notably, 37 and 62 of these targets overlapped with DN-related targets from GeneCards, DisGeNET, and OMIM. Protein-protein interaction (PPI) network analysis revealed hub genes, including PPARG, TLR4, and TP53, as critical mediators. Gene Ontology (GO) enrichment analysis revealed involvement in biological processes such as collagen metabolic process for EPA and regulation of inflammatory response for DHA, while KEGG pathway analysis highlighted the modulation of PPAR signaling, the renin-angiotensin system, and the AGE-RAGE signaling pathway. Molecular docking confirmed favorable binding affinities of EPA and DHA to key targets such as PPARG (-8.04 kcal/mol for DHA) and PPARD (-8.11 kcal/mol for EPA). These findings suggest that EPA and DHA may mitigate DN-associated inflammation through multi-target and multi-pathway interactions, positioning them as potential supplementary therapeutic agents.

Keywords: fish oil, inflammation, network pharmacology, supplements, sustainable medicine.

1. INTRODUCTION

Approximately 30–40% of diabetic patients have diabetes nephropathy (DN), a common complication of diabetes mellitus, and is the most common cause of end-stage renal disease worldwide [1], [2]. Although DN wasn't historically viewed as an inflammatory disease, recent research has demonstrated that kidney inflammation contributes significantly to the onset and progression of DN. The various disturbances seen in diabetic kidneys, whether metabolic, biochemical, or related to blood flow, may trigger inflammation, which could be a crucial mechanism in this disease process [3]. Current treatments, such as angiotensin-converting enzyme inhibitors and glycemic control, effectively manage certain aspects of DN, including blood pressure and glucose levels; however, they may not fully address the underlying inflammatory processes, highlighting the potential for complementary approaches to enhance inflammation management.

Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), two omega-3 polyunsaturated fatty acids, have drawn interest due to their renoprotective and anti-inflammatory properties [4], [5], [6]. As essential fatty acids that must be obtained through diet due to the lack of

delta-12 desaturase in humans, EPA and DHA are abundant in fish oils, including those derived from Patin (*Pangasius micronemus*), a freshwater fish widely cultivated in Southeast Asia. Patin fish oil (PFO) serves as a sustainable and cost-effective source of these PUFAs, making it a viable option for dietary supplementation. Patin fish oil was chosen in this study because it has a fatty acid profile that remains rich in DHA and EPA, although not as high as snakehead. However, it is much more widely available in industry and households, and is used as a major food source [10]. As supplements, EPA and DHA may support the management of inflammation in DN by modulating pro-inflammatory cytokine production, reducing oxidative stress, and promoting the synthesis of specialized pro-resolving mediators (SPMs) [7], such as resolvins and protectins, which could help alleviate glomerular dysfunction and proteinuria. It is yet unclear exactly which signalling pathways and biological processes underlie these therapeutic effects.

The advent of network pharmacology has significantly transformed the study of complex biological processes underlying natural product medicines, complemented by molecular docking analyses. This advanced computational method clarifies the complex interactions between bioactive substances and their molecular targets within biological networks by integrating systems biology concepts with bioinformatics algorithms [8]. Network pharmacology provides comprehensive insights into the multifaceted effects of fish oil constituents by enabling the simultaneous identification of multiple therapeutic targets and related signaling pathways. Molecular docking simulations, on the other hand, allow for the atomic-level analysis of ligand-protein interactions, which validates anticipated molecular targets and offers mechanistic insights into possible therapeutic activities [9].

In this study, an integrated network pharmacology and molecular docking method was employed to elucidate the molecular mechanisms underlying the effects of fish oil components on nephropathy. In particular, the aims of this study are (1) to identify the therapeutically active fatty acid compositions, particularly EPA and DHA in *Pangasius micronemus* fish oil from the previous existing research studies, (2) to clarify the protein targets and signalling pathways implicated in diabetic nephropathy via network analysis, and (3) to estimate the binding interactions and affinities of the selected compounds to their predicted molecular targets using advanced docking simulations. This comprehensive research will improve our knowledge of the therapeutic potential of fish oil, especially EPA and DHA. It could lead to the development of more potent treatments for diabetic nephropathy. This study has limitations and provides recommendations for further research that includes molecular dynamics analysis so that dynamic interactions and the flexibility of proteins and ligands can be investigated further.

2. MATERIALS AND METHODS

2.1. Screening and analysis of fatty acids and diabetic nephropathy-related targets

Screening of fatty acids contained in Patin fish oil (*Pangasius micronemus*) was obtained from previous research [10]. The screening phase for the potential targets of EPA and DHA is searched by inserting SMILES of each obtainable from PubChem (https://pubchem.ncbi.nlm.nih.gov/) [11], into the Swiss Target Prediction database (http://www.swisstargetprediction.ch/), SEA database (https://sea.bkslab.org/), and SuperPRED database (https://prediction.charite.de/). Species "*Homo sapiens*" were selected in the SEA database and probability above 50% were selected in the SuperPRED database. The potential target obtained will be determined by nomenclature approval using UniProt Retrieve/ID Mapping (https://www.uniprot.org/) [12].

Information on the disease targets related to diabetic nephropathy was collected from the GeneCards database (https://www.genecards.org/), the DisGeNET database (https://www.disgenet.org/), and Online Mendelian Inheritance in Man (OMIM) (https://www.omim.org/). The keyword "diabetic nephropathy" was used to obtain diabetic nephropathy-related targets from each database. Duplicate results were eliminated after merging the desired results from the three databases. All potential targets of fatty acids (EPA and DHA) and

diabetic nephropathy were submitted to the Jvenn diagram tool (https://jvenn.toulouse.inrae.fr/app/index.html) to visualise the intersection [13].

2.2. Construction of the protein-protein interaction (PPI) network and hub targets

The overlapping targets of fatty acids and diabetic nephropathy were imported into the STRING database (https://cn.string-db.org/) for PPI analysis. The analysis was performed using the "*Homo sapiens*" organism, with a minimum required interaction score of 0.9. The results of the PPI network were visualized and analyzed using Cytoscape v3.10.1 software. The degree values in the PPI network were calculated using the CytoHubba plugin of Cytoscape v3.10.1 software to investigate the network's topological properties.

2.3. GO and KEGG enrichment pathway analysis

A potential target biological function study on the effects of EPA and DHA on diabetic nephropathy was conducted using SRPLOT (https://www.bioinformatics.com.cn/en). Gene Ontology (GO) analysis was used to screen biological processes (BP), cellular components (CC), and molecular functions (MF). In contrast, Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment pathway analysis was used to identify key signaling pathways involved in biological processes.

2.4. Molecular docking

The intersection of the top 10 hub targets and pathways related to DN (PPAR signalling pathway, renin-angiotensin system, and AGE-RAGE signalling pathway in diabetic complications) was selected for molecular docking. Seven core targets were identified: BCL2, NFKB1, MMP2, ACE, PPARG, PPARA, and PPARD. However, as NFKB1 is a transcription factor lacking a defined binding site, molecular docking was performed only on the remaining six targets: BCL2 (PDB: 2W3L), MMP2 (PDB: 7XJO), ACE (PDB: 1O86), PPARG (PDB: 7AWC), PPARA (PDB: 2ZNN), and PPARD (PDB: 3OZ0). and RCSB PDB (http://www.rcsb.org/) databases, respectively. Then, the 3D structure of the core targets was imported into BIOVIA Discovery Studio (v24.1.0.23298) to remove native ligands, dehydrate, and hydrogenate the primary target. The crystal structure of the active components (eicasapentaenoic acid and docosahexaenoic acid) and the 3D structure of the core targets were obtained from the PubChem (https://pubchem.ncbi.nlm.nih.gov/) and the structure of 'MOL2' was exported. The active components and hub genes were converted into pdbqt format using Autodock Tools (v1.5.7) software. Molecular docking of the active components with the core targets was performed using Autodock Tools (v1.5.7) to predict binding affinities and modes. Low RMSD (<2 Å) of the redocked ligand from the orientation of the cocrystallized ligand and the replication of observed contacts from the pdb structure validated the docking. The best docking pose results were visualized by BIOVIA Discovery Studio.

3. RESULTS AND DISCUSSION

3.1. Fatty acids screening

The fatty acid profile of Patin fish oil (PFO), derived from *Pangasius micronemus*, was analyzed and is presented in Figure 1 [10]. The results reveal a diverse composition of fatty acids (% relative), categorized into saturated, monounsaturated, and polyunsaturated fatty acids (PUFAs). Saturated fatty acids constituted 39.764% (r) of the total, with hexadecanoic acid (C16:0) being the most abundant at 22.814% (r), followed by octadecanoic acid (C18:0) at 5.464% (r). Monounsaturated fatty acids accounted for 37.680% (r) of the total, with oleic acid (C18:1) and nervonic acid (C24:1) representing significant contributions at 31.078% (r) and 3.011% (r), respectively. Among the PUFAs, which comprised 22.340% (r) of the total, linoleic acid (C18:2) was the predominant component at 14.703% (r), while docosahexaenoic acid (C22:6) and eicosapentaenoic acid (C20:5) were present at 2.076% (r) and 0.447% (r), respectively. These findings highlight the rich PUFA content, particularly EPA and DHA, in PFO, which are known for their anti-inflammatory properties. Freshwater fish species often have lipid profiles that are consistent with the domination of oleic acid among

monounsaturated fatty acids and hexadecanoic acid among saturated fatty acids [14]. At the same time, the moderate levels of EPA and DHA suggest that PFO could serve as a supplementary dietary source of omega-3 PUFAs, supporting its application in nutritional studies.

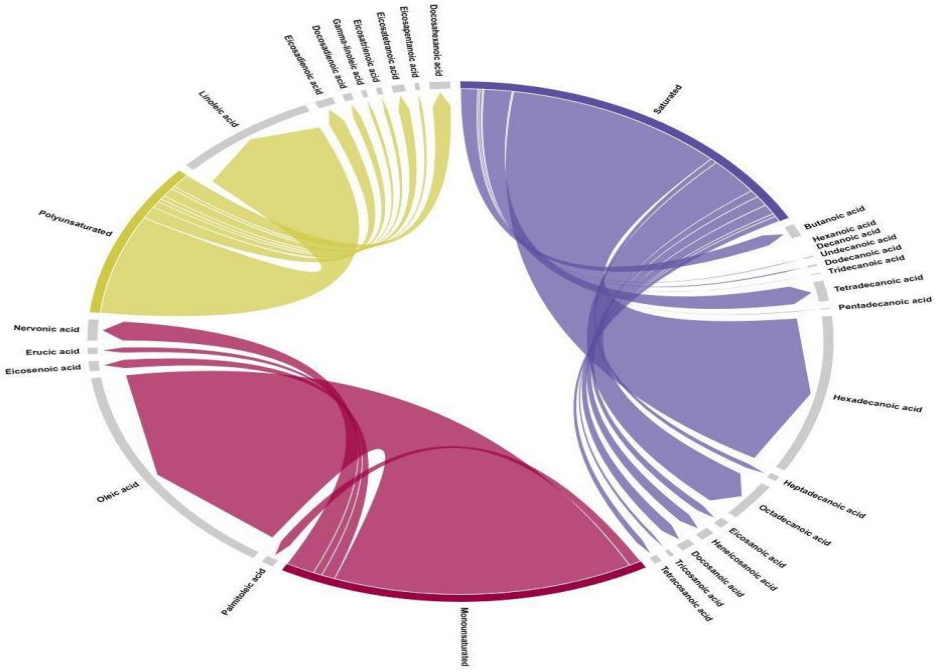


Figure 1. Fatty acid composition of PFO. The purple arrow represents saturated fatty acid, the red arrow represents monounsaturated fatty acid, and the yellow arrow represents polyunsaturated fatty acid.

3.2. Determination of fatty acids and diabetic nephropathy-related targets

After eliminating duplicates, 160 EPA targets and 185 DHA targets were obtained from three databases (Swiss Target Prediction, SuperPRED, and Similarity Ensemble Approach). Targets related to diabetic nephropathy from GeneCards, DisGeNET, and OMIM were also obtained, totaling 1630 targets. Ultimately, Venn diagrams with Jvenn were utilized, and 62 and 57 intersection targets were obtained as EPA and DHA targets against DN (Figure 2).

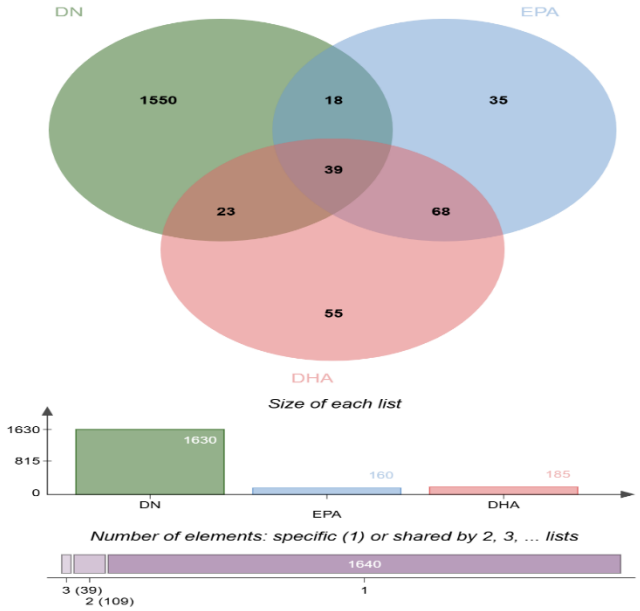


Figure 2. Venn diagram of common target genes between EPA, DHA, and DN.

3.3. PPI network analysis and hub genes

The STRING database was used to determine the protein-protein interaction (PPI), constructing 57 putative targets for EPA (Figure 3A) and 62 for DHA (Figure 3B). The PPI networks elucidate a protein's function by mapping its interactions within a complex web of molecular connections. These interactions are critical for predicting the function of target proteins and assessing the druggability of molecules [15]. We conducted a topological analysis of TSV files from the STRING database using Cytoscape v3.10.1 software with the CytoHubba plugin, identifying the top 10 hub genes based on degree for EPA and DHA-related targets (Figure 3C, D). PPARG, PPARA, PPARD, TLR4, TP53, HMGCR, ACE, and MMP2 are key targets shared by both EPA and DHA that contribute to the treatment of diabetic nephropathy.

This study's identification of PPARG, TLR4, and TP53 as major targets is consistent with earlier studies on diabetic nephropathy (DN) [16], [17], [18], [19], [20]. A key modulator of renal physiology and pathophysiology, PPARG agonists have protective effects against various kidney disorders, including DN. PPARG agonists may be more effective at slowing the progression of DN when used in conjunction with other renoprotective medications or lifestyle changes than when used alone [21]. Furthermore, TLR4 expression is markedly increased in diabetic patients with renal failure, which probably aggravates insulin resistance in type 2 diabetes and advances DN [22]. Recent research has emphasised the harmful involvement of the TP53 gene, which encodes the tumour suppressor protein p53, in diabetes complications, including DN [23].

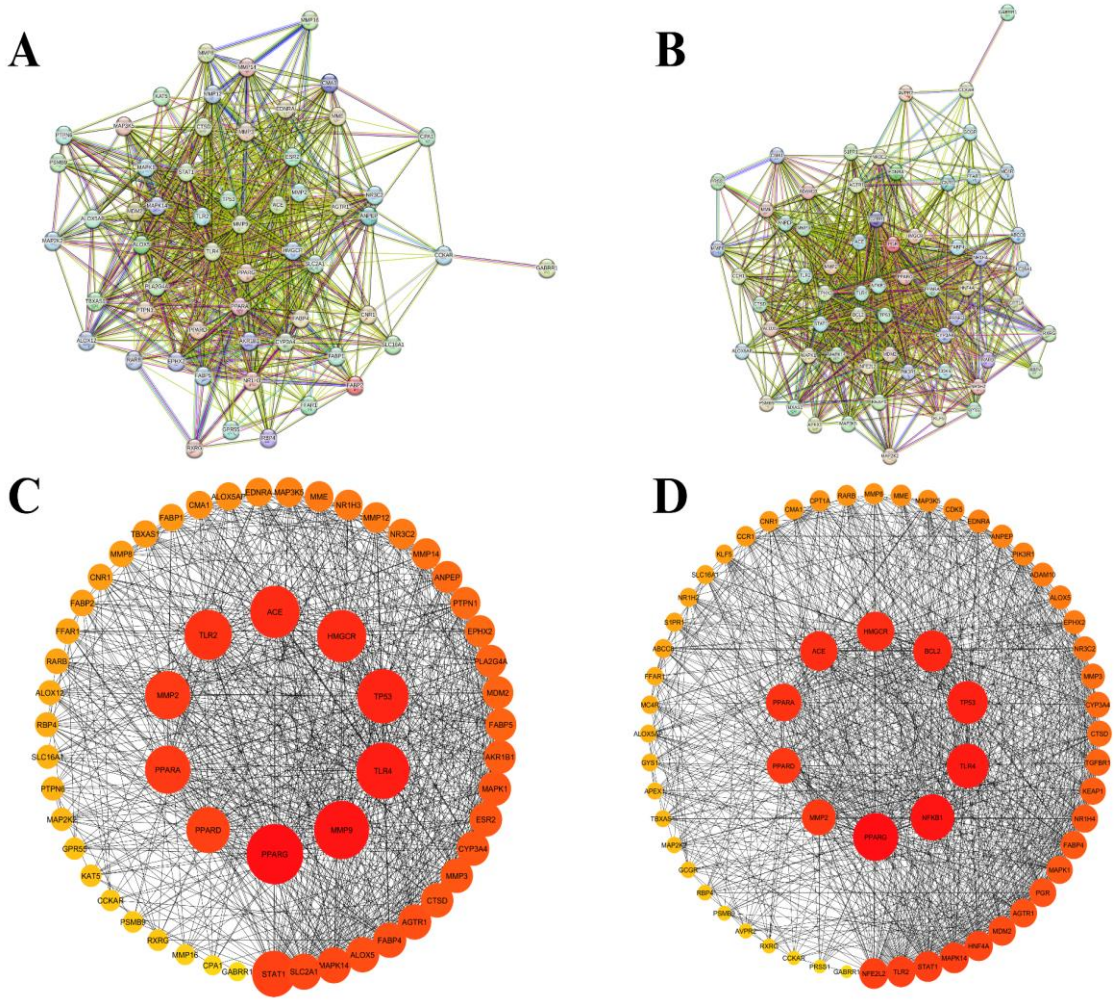


Figure 3. Analysis results of the PPI network EPA (A) and DHA (B), and hub targets of EPA (C) and DHA (D) visualized with Cytoscape. The colour and size of each node represent the degree.

3.4. Gene ontology and KEGG pathway enrichment analysis

To gain deeper insight into the action principle of EPA and DHA in treating DN, we conducted a functional enrichment analysis for the 57 and 62 obtained targets, resulting in 1,118 biological processes (BP), 45 cellular components (CC), and 114 molecular functions (MF) for EPA (p-value < 0.05) and 1,318 biological processes (BP), 42 cellular components (CC), and 136 molecular functions (MF) for DHA (p-value < 0.05). To visualize the results, we created bar charts using SRPLOT, highlighting the top 10 most significantly enriched GO terms (Figure 4A, C). Based on EPA action in treating DN, targets for BP are mostly involved in muscle cell proliferation, fatty acid metabolic process, collagen catabolic and metabolic process, regulation of inflammatory response, etc. CC targets were primarily found in the tertiary granule lumen, ficolin-1-rich granule, etc. Targets for MF included nuclear receptor activity, ligand-activated transcription factor activity, metallopeptidase activity, etc.

Furthermore, GO terms in DHA show targets for BP mostly involved in hormone secretion, response to nutrient levels, hormone transport, etc. CC targets were primarily found in membrane rafts, membrane microdomains, membrane regions, etc. Targets for MF included ligand-activated transcription factor activity, DNA-binding transcription factor binding, nuclear receptor activity, etc. The KEGG analysis revealed that the key targets were associated with 111 pathways for EPA and 127 pathways for DHA, with the top 15 pathways displayed in a Sankey and a dot plot based on their gene ratio, counts, and p-value (Figure 4B, D). The main KEGG pathways targeted by EPA against DN included the PPAR signalling pathway, efferocytosis, endocrine resistance, bladder cancer, and the renin-angiotensin system, among others. The main KEGG pathways targeted by DHA against DN included lipid and atherosclerosis, efferocytosis, sphingolipid signalling pathway, hepatitis B, and AGE-RAGE signalling pathway in diabetic complications, among others.

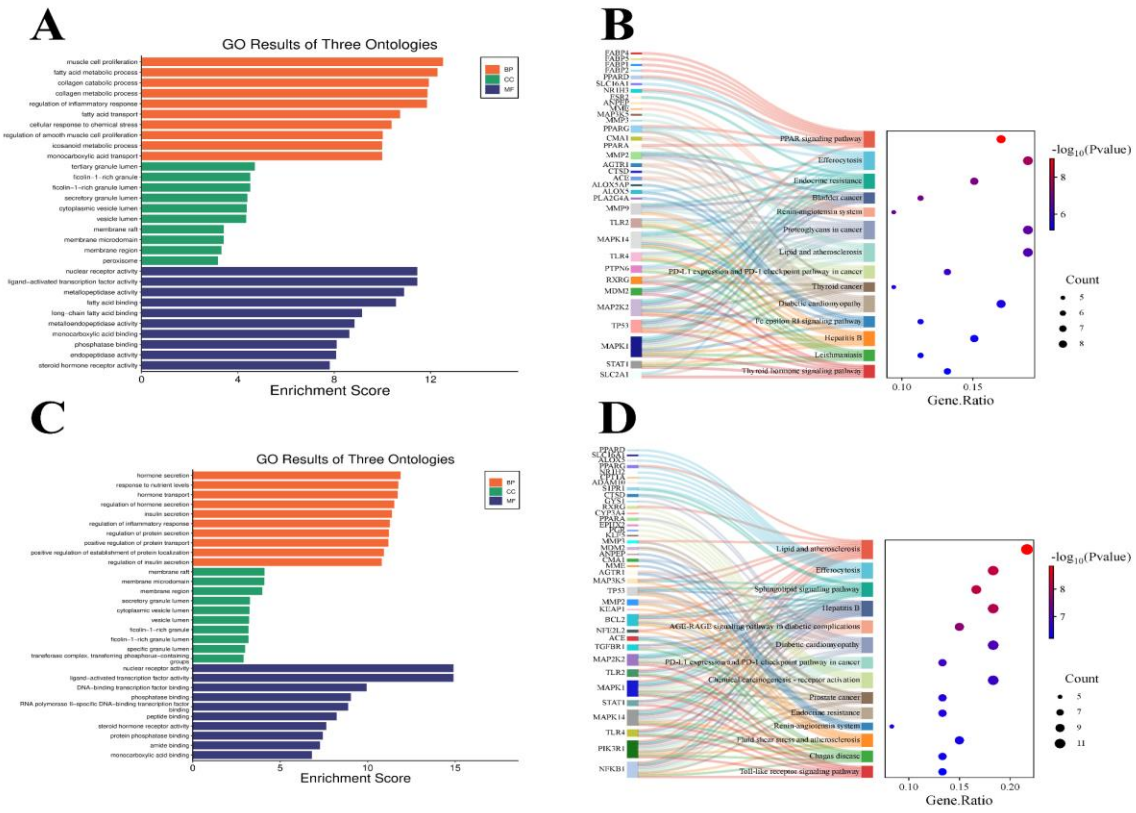


Figure 4. Gene ontology of biological process, cellular component, and molecular function of EPA (A) and DHA (C). The Sankey dot plot presents the KEGG enrichment analysis of the overlapped target EPA (B) and DHA (D)

3.5. Network construction of EPA and DHA-Targets-DN

Subsequently, the FA-targets-DN-BP-Pathway interaction network was built, comprising 117 nodes (containing 19 biological processes, 15 pathways, 80 target genes, 1 disease, and 2 active constituents) and 541 edges. According to network pharmacology findings, the network's active constituents (EPA and DHA) were associated with most target genes. As seen in Figure 5, the network was generated using Cytoscape 3.10.1. Table 1 provides a detailed breakdown of the top 3 BP and the pathway related to DN and associated with its overlapped targets.

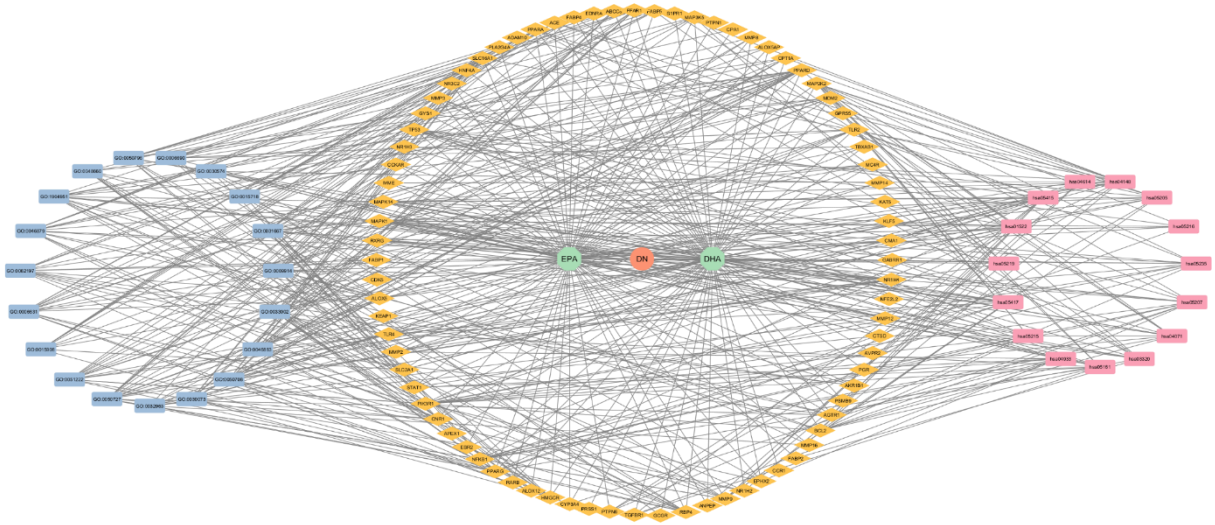


Figure 5. Network construction of EPA and DHA-Target-DN-BP-Pathway. The blue nodes represent BP, the yellow nodes represent the target genes, and the red nodes represent the KEGG pathway

Table 1. The top 3 biological processes and pathways related to DN.

Biological Processes		Pathway	
Term	**Genes**	**Term**	**Genes**
Regulation of inflammatory response (GO:0050727)	MMP9, NR1H3, PPARG, AGTR1, TLR4, MAPK14, MMP3, FABP4, MMP8, CNR1, TLR2, ALOX5, PPARA, CMA1, PPARD	PPAR signaling pathway (hsa03320)	FABP2, FABP1, NR1H3, FABP5, PPARG, FABP4, PPARA, PPARD, RXRG
collagen metabolic process (GO:0032963)	MMP9, MMP14, MMP12, MMP16, PPARG, CTSD, MMP2, MMP3, MMP8, PPARD	Renin-angiotensin system (hsa04614)	ACE, AGTR1, MME, CMA1, ANPEP
cellular response to chemical stress (GO:0062197)	AKR1B1, SLC2A1, MMP9, FABP1, PPARG, MMP2, TLR4, MAPK1, MMP3, TP53, ALOX5, MAP3K5, MDM2	AGE-RAGE signalling pathway in diabetic complications (hsa04933)	TGFBR1, BCL2, NFKB1, PIK3R1, AGTR1, MMP2, MAPK14, STAT1, MAPK1

3.6. Molecular docking analysis

To enhance the validity of our network pharmacology analysis, we conducted molecular docking analysis. Table 2 presents the molecular docking results, expressed as binding affinity scores in kcal/mol, for various ligands against a panel of diabetic nephropathy-related proteins, including BCL2, MMP2, ACE, PPARG, PPARA, and PPARD. The ligands evaluated include the test compounds eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), alongside other natural ligands such as tetrahydroisoquinoline amide, lisinopril, rosiglitazone, etc.

MMP2:
Re-docking: 2.12 A (run 7)
Gridbox: n points in x, y, z dimensions (40, 40, 40) and x, y, z center (41,069; -61,306; 19.645)

BCL2:
Re-docking: 0.85 A (run 9)
Gridbox: n points in x, y, z dimensions (38, 38, 36) and x, y, z center (38,418; 26,949; -12,545)

ACE:
Re-docking: 1.94 A (run 1)
Gridbox: n points in x, y, z dimensions (38, 38, 36) and x, y, z center (40,899; 32,391; 47,285)

PPARG:
Re-docking: 0.75 A (run 10)
Gridbox: n points in x, y, z dimensions (40, 40, 40) and x, y, z center (41,391; 3,223; 82,402)

PPARA:
Re-docking: 1.27 A (run 8)
Gridbox: n points in x, y, z dimensions (40, 40, 40) and x, y, z center (11,98; 4,435; -7,652)

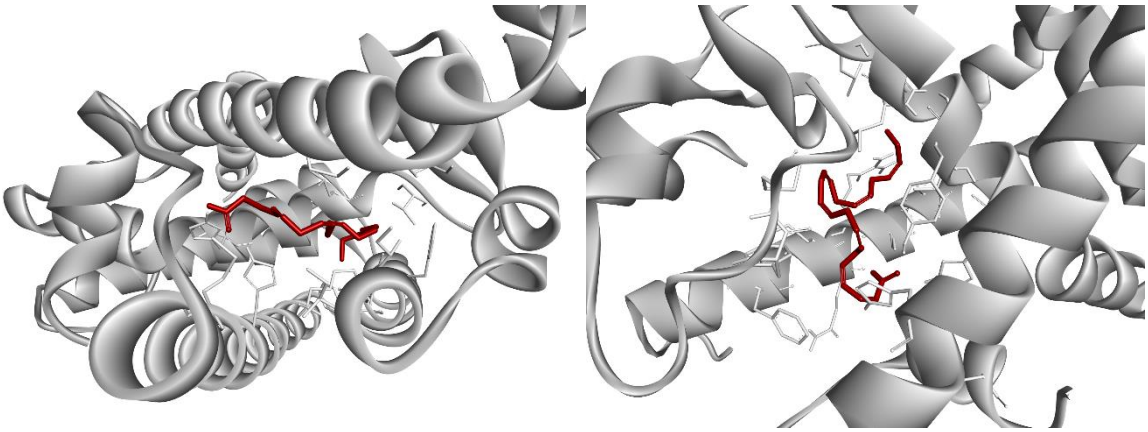
PPARD:
Re-docking: 0.81 A (run 7)
Gridbox: n points in x, y, z dimensions (40, 40, 40) and x, y, z center (1,304; -15,909; 47,746)

Table 2. Docking score of diabetic nephropathy-related proteins

Drug/Compounds	Docking score (kcal/mol)					
	BCL2	**MMP2**	**ACE**	**PPARG**	**PPARA**	**PPARD**
EPA	-5.42	-1.60	-5.76	-7.20	-5.99	-8.11
DHA	-4.86	-1.92	-5.66	-8.04	-7.36	-7.83
Phenyl Tetrahydroisoquinoline amide	-10.62	-	-	-	-	-
Aryloxyphenyl–Heptapeptide	-	-3.43	-	-	-	-
Lisinopril	-	-	-6.62	-	-	-
Rosiglitazone	-	-	-	-9.53	-	-
TIPP-703	-	-	-	-	-11.41	-
2-[4-[[(1S)-1-[(2,4-dichlorophenyl)carbamoyl]-1,3-dihydroisoindol-2-yl]methyl]-2-methylphenoxy]acetic acid	-	-	-	-	-	-11.68

EPA and DHA exhibit moderate binding affinities across the tested proteins. Specifically, EPA shows binding affinity ranging from -1.60 kcal/mol (MMP2) to -8.11 kcal/mol (PPARD), with the strongest interaction observed with PPARD (Figure 6A). DHA displays binding energies from -1.92 kcal/mol (MMP2) to -8.04 kcal/mol (PPARG), also demonstrating a notable affinity for PPARG (Figure 6B). DHA makes an H-bond interaction with Tyr 423, Ser 289 and His323; hydrophobic interactions at Arg288, Cys285 and Leu330. Prior investigations utilizing the PPARG protein structure revealed that the binding location between the PPARG protein and the ligand features a canonical orthosteric contact with Tyr473, located inside the activation function of helix 12, alongside an allosteric interaction with Arg288 [25]. These results suggest that both omega-3 fatty acids interact preferentially with PPAR family members (PPARG, PPARA, PPARD), which

are known to regulate lipid metabolism and inflammation, key processes in diabetic nephropathy [24]. In contrast, their affinities for BCL2, MMP2, and ACE are relatively weaker, indicating limited interaction with these targets under the docking conditions.



(a) (**b**)

Figure 6. Docking pose of EPA-PPARD (**a**) and DHA-PPARG (**b**).

Table 3 presents the amino acid residues involved in the interactions between proteins and ligands. In the BCL2 protein, all amino acid residues of EPA and DHA show similarity to those of the native ligand; however, a difference is observed between the EPA and DHA residue (Asn102) and the native ligand residue (Arg88). For the MMP2 protein, the amino acid residues of EPA and DHA exhibit similarity to those of the native ligand, specifically Tyr74, Gly73, Glu3, and Leu82. In contrast, the ACE protein demonstrates differences in amino acid residues between EPA and DHA and the native ligand, namely at Gln281, His387, Val518, Val380, Phe512, and Val351.

Table 3. Amino acid residue.

Protein	Ligand	Key Residue/Amino Acid Residue
BCL2	EPA	Asn102, **Gly104**, **Arg105**, **Tyr67**, **Val92**, **Met74**, **Glu95**, **Phe112**, **Leu96**, **Phe71**, **Ala108**, **Phe63**
	DHA	Asn102, **Arg105**, **Phe112**, **Val92**, **Leu96**, **Met74**, **Ala108**, **Phe71**, **Phe63**, **Asp70**, **Tyr67**
	Phenyl Tetrahydroisoquinoline amide	Arg88, Val92, Phe112, Met74, Tyr67, Phe71, Asp70, Phe63, Arg105, Gly104, Leu96, Ala108, Glu95
MMP2	EPA	**Tyr74**, **Glu3**, His85, Leu6, **Leu82**, Pro75, **Gly73**, Asp72
	DHA	**Tyr74**, His85, Leu6, **Leu82**, Pro75
	Aryloxyphenyl–Heptapeptide	Tyr74, Gly73, Glu3, Leu82, Pro85
ACE	EPA	**Lys511**, **Tyr520**, Gln281, **Tyr523**, **His353**, **His383**, His387, **His513**, Val518, Val380, Phe512
	DHA	**Lys511**, **Tyr520**, Gln281, **Tyr523**, **Glu384**, **Glu411**, **His353**, **His383**, **His513**, Val351, Val518, Phe457, Phe512
	Lisinopril	Tyr520, His353, His513, Tyr523, Glu384, Glu411, Lys511, His383, Ala354
PPARG	EPA	**His323**, **Ser289**, **Gln286**, **Tyr473**, **Cys285**, Met364, **Leu330**, **Leu341**, Leu469, Phe282, Phe363, Leu465, Lys367, **Ile341**, **Ile281**, **Ile326**, **Gly284**, **Arg288**, Leu333, **Leu339**, Tyr327, Met329, Ala292

	DHA	**Tyr473**, **Ser289**, **His323**, **Cys285**, Met364, Phe282, Phe363, **Leu330**, Leu333, **Leu339**, Leu465, Leu469, Met329, Ala292, **Arg288**, **Ile326**
	Rosiglitazone	Tyr473, Ser289, His323, His449, Gln286, Cys285, Met348, Ile341, Ile326, Leu330, Leu339, Val339, Leu344, Ile281, Leu341, Gly284, Arg288, Val348
PPARA	EPA	Thr279, **Cys276**, Ala333, **Leu321**, **Met330**, **Met355**, Leu344, **Leu334**, Val332, Ile339, Ile354, Lys358, **Phe318**, **Ser280**, Tyr314, **Gln277**, Val444, Tyr464, Leu456, Leu460
	DHA	Ala333, **Met330**, Val332, Thr279, **Leu321**, Ala334, Ile339, Ile354, Ile364, **Met325**, **Met355**, Met358, Leu344, Leu460, Leu456, **Gln277**, His440, **Ser280**, **Phe318**, Phe273, Tyr314, Tyr464, **Ala250**, Val444, **Cys276**
	TIPP-703	Met330, Cys276, Ser280, Phe318, Leu321, Met325, Val255, Cys255, Thr279, Ala332, Leu334, Val331, Ile317, Ile241, Leu241, Gln277, Glu277, Ala250, Ile358, Met355, Phe359
PPARD	EPA	**His323**, **His449**, **Tyr473**, **Cys285**, **Phe282**, **Phe363**, Met453, **Met364**, Ile363, Ile364, **Leu330**, Leu339, Leu445, **Val324**, Val327, Val334, Ala331, Ala337, Ala371, Lys367, Ala370
	DHA	Ala342, Arg284, Ile364, Leu353, **Leu330**, Leu339, Leu445, Phe327, Phe370, **Val281**, Val334, Val348, Val341
	2-[4-[[(1S)-1-[(2,4-dichlorophenyl)carbamoyl]-1,3-dihydroisoindol-2-yl]methyl]-2-methylphenoxy]acetic acid	His449, Tyr473, His323, Thr289, Gln286, Cys285, Phe282, Phe363, Ile341, Leu330, Val339, Val324, Met364, Leu469, Ala348, Val281, Arg288, Val288, Ala285, Phe287, Val284

The docking scores reflect the stability of ligand-protein complexes, with more negative values indicating stronger binding. These findings highlight the potential of EPA and DHA, derived from natural sources like Patin fish oil, to modulate diabetic nephropathy-related pathways, particularly through PPAR-mediated mechanisms. Further experimental validation is required to confirm these interactions and their biological relevance.

4. CONCLUSION

This bioinformatics study elucidates the molecular mechanisms by which EPA and DHA from Patin fish oil exert anti-inflammatory effects in DN. Employing network pharmacology, we identified key targets, including PPARG, TLR4, and TP53, and demonstrated their interactions within a protein-protein interaction network. Enrichment analyses revealed that EPA and DHA modulate critical pathways such as PPAR signaling, renin-angiotensin system, and AGE-RAGE signaling, alongside biological processes like collagen metabolic process and regulation of inflammatory response, all of which are implicated in DN progression. Molecular docking validated these findings, showing significant binding affinities of EPA and DHA to targets such as PPARG and PPARD. Collectively, these results underscore the therapeutic potential of EPA and DHA as supplementary agents for managing DN by targeting inflammatory and fibrosis. Further experimental and clinical studies are warranted to confirm these interactions and assess their practical efficacy.

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