International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 15, Special Issue 2, 2023

Original Article

IN SILICO ASSESSMENT OF AMELIORATIVE EFFECTS OF POLYUNSATURATED FATTY ACID (PUFAS) FROM *NAVICULA SALINICOLA* AS AN INHIBITOR OF BENIGN PROSTATE HYPERPLASIA

ELLIN FEBRINA[1*], ANNE YULIANTINI[2], DEWI KURNIA[2], AIYI ASNAWI[2]

[1]Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Padjadjaran, Jatinangor-45363, West Java, Indonesia. [2]Department of Pharmacochemistry, Faculty of Pharmacy, Universitas Bhakti Kencana, Bandung-40617, West Java, Indonesia
[*]Corresponding author: Ellin Febrina; [*]Email: ellin.febrina@unpad.ac.id

Received: 29 Aug 2023, Revised and Accepted: 26 Sep 2023

ABSTRACT

Objective: Benign prostatic hyperplasia (BPH) is a prevalent, non-cancerous condition affecting aging men worldwide. As an alternative approach to conventional treatment options, polyunsaturated fatty acids (PUFAs) have gained attention for their potential therapeutic effects on various health conditions. This study investigated the interaction of PUFAs obtained from *Navicula salinicola* with the macromolecule associated with BPH, represented by STAT3, that is involved in the androgen signaling pathway in BPH (PDB ID 6NJS), using molecular docking simulations.

Methods: The docking simulations revealed the interaction patterns and binding affinities of 14 PUFAs with the amino acid residues of STAT3. The calculated binding energies and inhibition constants provided insights into the potential inhibitory effects of PUFAs on BPH.

Results: Results indicated that γ-linolenic acid exhibited a strong binding affinity, forming hydrogen bonds with ARG609 and hydrophobic interactions with VAL637 and PRO639, highlighting its potential as a potent inhibitor. Docosahexaenoic acid also showed favorable interactions with ARG609 and hydrophobic residues, suggesting its potential therapeutic relevance.

Conclusion: γ-Linolenic acid from *N. salinicola* exhibited a strong molecular interaction with STAT3.

Keywords: Benign prostatic hyperplasia (BPH), Inhibitor, Molecular docking, *Navicula salinicola*, Polyunsaturated fatty acids (PUFAs)

DOI: https://dx.doi.org/10.22159/ijap.2023.v15s2.16 Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

Benign prostatic hyperplasia (BPH) is a prevalent, non-cancerous condition that affects a significant number of aging men worldwide [1]. BPH involves the enlargement of the prostate gland, leading to the compression of the urethra and subsequent urinary symptoms such as difficulty in urination, increased frequency, and nocturia [2]. Despite being a non-life-threatening condition, BPH can significantly impact the quality of life and well-being of affected individuals. Currently, available treatment options for BPH include medications, minimally invasive procedures, and surgery [3]. However, minimally invasive surgery is still surgery, and there are risks of complications such as bleeding, infection, and injury to organs [4, 5]. Therefore, there is a growing interest in exploring alternative and potentially more effective therapeutic approaches.

The signal transducer and activator of transcription 3 (STAT3) is a protein that plays a role in the development of BPH. STAT3 is involved in the activation of NF-κB in the prostate as a result of high-fat diet feeding, leading to inflammation [6]. Constitutive activation of STAT3 causes growth dysregulation and resistance to apoptosis through changes in gene expression, leading to hyperproliferation of prostate tissue [7]. STAT3 is involved in the androgen signaling pathway in BPH [8]. The aberrant activation of STAT3 leads to increased cell proliferation and reduced cell apoptosis, resulting in BPH development [9]. The EGF/STAT3 signaling pathway is involved in the development of BPH, and qianliening capsule (QC) treatment may significantly decrease the serum level of EGF in BPH rats as well as downregulate the mRNA and protein levels in STAT3 [10].

Among the numerous natural compounds investigated for their potential health benefits, PUFAs have garnered attention due to their diverse physiological roles and potential therapeutic effects [11]. PUFAs, particularly omega-3 and omega-6 fatty acids, are essential nutrients obtained through diet and have been extensively studied for their cardiovascular, anti-inflammatory, and anticancer properties [12]. Researchers have also begun to investigate their potential impact on prostate health, particularly in the context of BPH [13].

In recent years, a specific microalgae called *N. salinicola* has emerged as a promising source of bioactive compounds, including various PUFAs [14]. This microorganism thrives in saline environments and has been found to contain a rich reservoir of valuable lipids with potential health benefits [15]. *N. salinicola* is a species of diatom, a type of unicellular algae. Diatoms are known to be a potent source of PUFAs, which are essential for good health in humans and many animals [16]. *N. salinicola* has been studied for its fatty acid profile, and its potential as a source of PUFAs has been investigated [17]. Researchers have turned their attention to *N. salinicola* and its PUFA content to explore their therapeutic potential in various health conditions, including BPH.

The PUFAs found in *N. salinicola* have been of particular interest due to their potential as inhibitors of benign prostate hyperplasia [18]. Benign prostate hyperplasia is a non-cancerous enlargement of the prostate gland that can cause urinary problems in men. PUFAs have been studied for their potential to inhibit the growth of prostate cells and reduce the risk of benign prostate hyperplasia [19]. Most data regarding the effects of high dietary n-6 PUFA are positively associated with prostate cancer incidence. However, the ratio of *n*-6 to *n*-3 fatty acids may be more important than the absolute amount of n-3 PUFA [20]. PUFAs are associated with a reduced risk of several types of carcinogenesis, including prostate cancer. However, this depends on numerous factors, including the source of omega-3 PUFAs. The consumption of a high-fat diet and obesity cause a reduction in testosterone and promote prostatic changes such as prostatitis, BHP, and high-grade prostatic intraepithelial neoplasma (HGPIN) until cancer [21].

Despite these exciting developments, there remains a significant research gap about the molecular mechanisms underlying the potential inhibitory effects of PUFAs from *N. salinicola* on BPH [17]. Omega-3 PUFAs have been studied for their potential role in the treatment of benign prostatic hyperplasia (BPH). A study evaluated the effects of concentrated long-chain omega-3 polyunsaturated fatty acid (LCn3) supplementation on prostate cancer-specific quality of life in men treated by radical prostatectomy. The study found that LCn3 supplementation improved urinary incontinence and bowel function [22]. Another study investigated the effects of combination therapy with omega-3 fatty acids, tamsulosin, and finasteride in the treatment of men with lower urinary tract symptoms (LUTS) and BPH. The study found that the combination

therapy improved LUTS and BPH symptoms [23]. Omega-3 PUFAs have been shown to reduce blood pressure and vasodilation, which may be beneficial for men with BPH [24]. Supplementation of PUFAs and aerobic exercise have been shown to improve functioning, morphology, and redox balance in prostate-obese rats [21]. A study showed significantly lower omega-3 levels in men without prostate cancer, suggesting that omega-3 PUFAs may play a role in prostate cancer prevention [25]. While there have been some preliminary studies indicating the possible benefits of PUFAs in managing BPH, the exact molecular pathways and interactions between these bioactive compounds and prostate cells remain largely unexplored. Therefore, the present study aims to address this research gap through molecular docking analyses to gain deeper insights into the potential interactions between PUFAs from *N. salinicola* and the key molecules involved in BPH development.

Molecular docking is a computational technique that can be used to predict the binding affinity of a ligand to a receptor. In the field of BPH, molecular docking has been utilized in various research designs to investigate the potential of therapeutic targets and inhibitors for the treatment of BPH. Molecular docking can be used to identify potential therapeutic targets for the treatment of BPH. "A functional genomics pilot study" utilized molecular docking to identify alternative Chinese herbal agents for the treatment of BPH [26]. Molecular docking can be employed to investigate the binding interactions between ligands and receptors in BPH [2]. Molecular docking can be used to identify potential inhibitors for the treatment of BPH [27].

Molecular docking simulations were used to study the interactions between PUFAs from *N. salinicola* and the selected target molecules involved in BPH pathogenesis. These simulations will predict and analyze the binding affinities and binding modes of PUFAs with the target molecules, providing insights into potential inhibitory effects.

MATERIALS AND METHODS

Materials

The personal computer was equipped with an Intel® CoreTM i7-7200U CPU at 2.50 GHz (4 CPUs at 2.7 GHz), 20 GB of RAM, and Windows 10 Pro 64-bit for molecular docking simulation.

Preparation of ligand structures

The chemical structures of PUFAs (table 1), in a *. sdf format file were obtained from the PubChem substance and compound databases (https://pubchem.ncbi.nlm.nih.gov/) [28]. The optimized 3D structures were converted using Discovery Studio Visualizer into *. pdb file formats for further use in molecular docking simulations.

Preparation of macromolecular structures

The crystal structure of the signal transducer and activator of transcription 3 (STAT3) (PDB ID 6NJS) along with the native ligand of SD36 ([(2-{[(5S,8S,10aR)-3-acetyl-8-{(2S)-5-amino-1-[(diphenylmethyl)amino]-1,5-dioxopentan-2-yl}carbamoyl)-6-oxodecahydropyrrolo[1,2-a][1,5]diazocin-5-yl]carbamoyl}-1H-indol-5-yl)(difluoro)methyl]phosphonic acid) [29] was downloaded from the Protein Data Bank. Water molecules, co-crystallized ligands, and other heteroatoms were removed from the 6NJS structure. Only the protein chain(s) of B relevant to the study were retained. The 6NJS structure was checked for missing atoms or residues. AutoDock Tools was used to add any missing hydrogen atoms and fill gaps in the protein chain.

Validation of molecular docking

Autodock 4.2 simulations were initiated to dock native ligand (ID SD36) onto the active site of the macromolecule (6NJS). A grid box was created around the native ligand of the macromolecule of the signal transducer and activator of transcription 3 (STAT3) (PDB ID 6NJS) to define the region where the native ligand would be docked. The grid dimensions were adjusted to cover the entire binding pocket while considering computational resources. Appropriate atom types, charges, and torsional information were assigned to the ligands. The docking parameters, such as the search algorithm (genetic algorithm), docking runs, and population size, were set up to customize the docking simulations. Autodock explored different conformations and orientations of the ligands within the binding site [30].

Simulation of molecular docking

Simulation of molecular docking was carried out using Autodock 4.2. The ligand structures of PUFAs prepared in the earlier steps were loaded into Autodock. Appropriate atom types, charges, and torsional information were assigned to the ligands. The grid box was created based on the grid box obtained from the re-docking results. The docking parameters, such as the search algorithm (genetic algorithm), docking runs, and population size, were set up to customize the docking simulations. The results of the docking experiment using Discovery Studio Visualizer were analyzed and visualized [31].

Analysis of docking results

The output files (*. dlg) generated by Autodock, containing docking scores, ligand poses, and interaction energies, were saved. By using a Discovery Studio Visualizer, the 6NJS macromolecular structure and the docked ligand poses were loaded into the program. The docking poses of each ligand within the binding pocket of the signal transducer and activator of transcription 3 (STAT3) were visualized and inspected. The binding interactions, such as hydrogen bonds, hydrophobic interactions, and pi-stacking, between the ligands and the amino acid residues of signal transducer and activator of transcription 3 (STAT3) were analyzed (for details, see [32–34]).

RESULTS

Validation of molecular docking procedures

In this study, Autodock 4.2 was used to explore the molecular interactions between PUFAs from *N. salinicola* and the macromolecule associated with benign prostatic hyperplasia (BPH), represented by the signal transducer and activator of transcription 3 (STAT3) (PDB ID 6NJS). Before docking, the macromolecule was processed to remove water molecules and any other heteroatoms while retaining the relevant protein chains associated with BPH. By processing the macromolecule in this way, it is possible to obtain a more accurate prediction of the interaction between the ligand and the macromolecular target. This is important for drug discovery and other applications that require understanding the binding affinity and specificity of a ligand to its target.

The grid parameters play a crucial role in defining the search space for docking simulations. The npts values of 40 Å, 56 Å, and 40 Å represent the number of grid sizes in the x, y, and z dimensions, respectively. The grid center, specified as (13.498, 54.118, and 0.1) Å, defines the center coordinates of the grid box within the macromolecule. The selected grid center should encompass the active site or binding pocket of the macromolecule to ensure that potential binding interactions are accurately explored during docking simulations. Grid data file was used to define the interaction potentials and polarizabilities of the receptor atoms. The spacing of 0.375 Å between the grid points determined the resolution of the grid, influencing the accuracy of the docking results. A smaller spacing can yield more precise results, but it requires higher computational resources. In this study, the chosen grid spacing balances accuracy and computational efficiency.

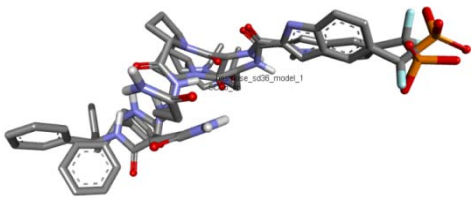


Fig. 1: Overlapping of the native ligand (SD36) model structure from the best pose resulting from re-docking against the native ligand (SD36) crystal structure of the 6NJS macromolecule

The root-mean-square deviation (RMSD) value of 1.541 from the reference structure shows that the ligand poses produced by the docking simulations were a little different from the known binding conformation of the reference molecule (fig. 1). This deviation is

within an acceptable range of 2.0 Å, considering the inherent flexibility of both the ligand and the receptor during docking simulations. The RMSD value confirms the reliability of the docking methodology used in this study.

The molecular interactions between SD-36 and the macromolecule of signal transducer and activator of transcription 3 (STAT3) (PDB ID 6NJS) were successfully validated using Autodock 4.2. The RMSD value indicated that the docking simulations generated ligand poses in close agreement with the reference structure. The carefully chosen grid parameters and atom types ensured an accurate representation of the binding site and intermolecular interactions. This validation step supports the reliability and robustness of the docking methodology used in this study and provides a strong foundation for the subsequent analysis and interpretation of the docking results. The amino acid residues Glu-638 and Tyr-657 in the STAT3 SH2 domain have an important role in the activity of STAT3. These residues are involved in the binding of STAT3 to its target DNA and are potential targets for small-molecule inhibitors of STAT3 activity [35].

Binding energies

We further investigated the binding interaction energies of various PUFAs with the macromolecule associated with benign prostatic hyperplasia (BPH), represented by the PDB ID 6NJS. The interaction energies were calculated using Autodock 4.2, and the obtained binding energies and inhibition constants (Ki) for each PUFA are presented in table 1.

The binding energies represent the strength of the interactions between each PUFA and the active site of the macromolecule (6NJS). A higher binding energy indicates stronger and more favorable interactions, suggesting a higher likelihood of the PUFA forming stable complexes with the macromolecule [36]. Based on the binding energies, it can be seen that the native ligand (SD36) has the most favorable binding energy of-6.93 kcal/mol, indicating a strong and stable interaction with the macromolecule. This native ligand likely represents a biologically relevant compound involved in BPH regulation.

Among the PUFAs tested, γ-linolenic acid (-4.7 kcal/mol) and docosahexaenoic acid (-4.51 kcal/mol) also exhibit relatively high negative binding energies, suggesting that they have a strong affinity for the active site of 6NJS. This finding highlights their potential as potent inhibitors of BPH. On the other hand, several PUFAs, such as erucic acid, eicosatrienoic acid, and nervonic acid, show less favorable binding energies, indicating weaker interactions with the macromolecule.

Table 1: Interaction energies of polyunsaturated fatty acids (PUFAs) in benign prostatic hyperplasia (PDB ID 6NJS)

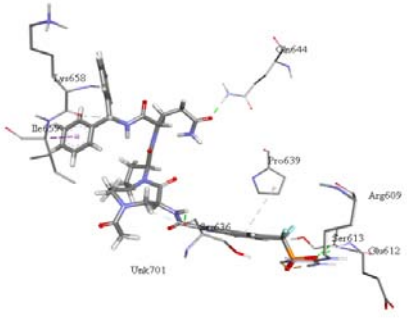
Code	Polyunsaturated fatty acids (PUFAs)	Binding energy, kcal/mol	Inhibition constant (Ki), mmol
SD36	Native ligand	-6.93	0.0083
ALA	α-Linoleic acid	-3.88	1.4400
ARA	Arachidonic acid	-3.94	1.3000
CHA	Cis-10-Heptadecenoic acid	-3.38	3.3200
DHA	Docosahexaenoic acid	-4.51	497.8400
EIA	Eicosatrienoic acid	-2.49	10.4500
ELA	Elaidic acid	-3.25	4.1400
EPA	Eicosapentaenoic acid	-3.16	4.8100
ERA	Erucic acid	-2.34	19.2300
GLA	γ-Linolenic acid	-4.70	0.3575
HDA	Hexadecadienoic acid	-3.54	2.5500
MYA	Myristoleic acid	-3.64	2.1600
NEA	Nervonic acid	-2.75	9.5900
OLA	Oleic acid	-3.14	4.9800
PAA	Palmitoleic acid	-3.59	2.3200

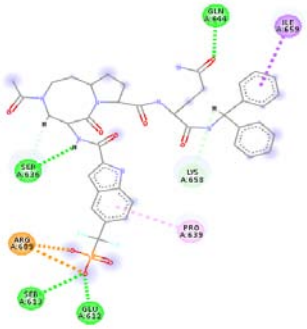
The inhibition constant (Ki) represents the concentration of the PUFA required to achieve 50% inhibition of the macromolecule's activity. A lower Ki value indicates a more potent inhibitor. The study found that γ-linolenic acid was a potent inhibitor with a Ki of 0.35746 mmol, followed by the native ligand (SD36) with a Ki of 0.00833 mmol.

It's worth noting that while some PUFAs show favorable binding energies and low Ki values, others exhibit weaker interactions. These results suggest that not all PUFAs may have significant inhibitory effects on BPH, and their potential as therapeutic agents should be further investigated in experimental settings.

Binding mode

Interaction analysis was performed to investigate the binding patterns between the top PUFAs and the amino acid residues of the macromolecule associated with BPH, represented by the PDB ID 6NJS. Analysis was conducted using Discovery Studio Visualizer, and the obtained data on hydrogen bonds (HB) and hydrophobic interactions (HI) between PUFAs and amino acid residues are presented in fig. 2. The interaction analysis provides valuable insights into the key residues involved in the binding of PUFAs to the active site of the macromolecule (6NJS), shedding light on the potential binding modes and stabilization of the complexes.





Native ligand (SD36)

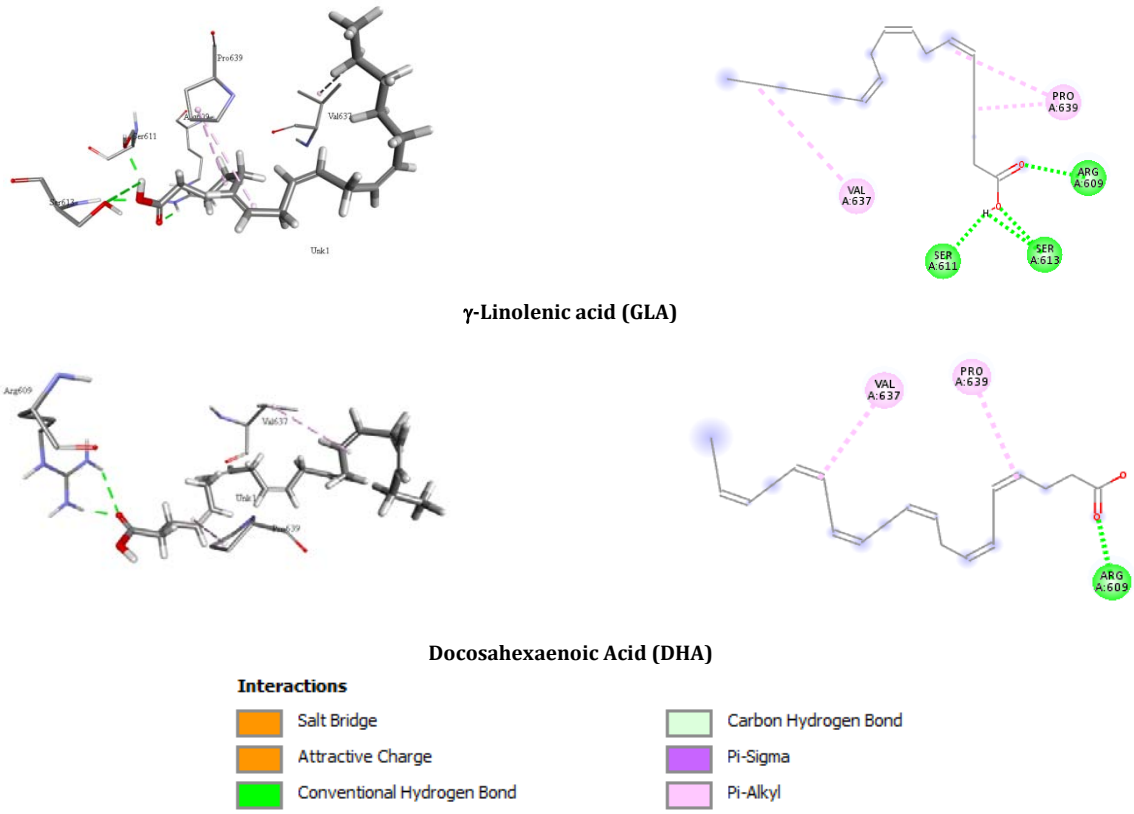


Fig. 2: A 2D and 3D view of the bond between top-two of the best-pose of PUFAs into amino acid residues of benign prostatic hyperplasia (PDB ID 6NJS)

Hydrogen bonds are important non-covalent interactions that play a pivotal role in ligand-receptor binding. The native ligand (SD36) forms four hydrogen bonds with specific amino acid residues of the macromolecule, namely GLU612, SER613, SER636, and GLN644. These hydrogen bonds contribute significantly to the stability of the ligand-receptor complex. Besides, γ-linolenic acid and docosahexaenoic acid also form a hydrogen bond pattern similar to SD-36. Tetapi, γ-linolenic acid, and docosahexaenoic acid also form hydrogen bonds with the same amino acid residue, ARG609. This indicated that interaction suggests a common binding mode for these PUFAs within the active site of the macromolecule.

Hydrophobic interactions involve *van der Waals* forces between non-polar regions of the ligand and the receptor. γ-Linolenic acid and docosahexaenoic acid engage in two hydrophobic interactions each, involving the amino acid residues VAL637 and PRO639. These hydrophobic interactions contribute to the stability of the complexes by minimizing unfavorable solvent exposure to hydrophobic ligands and receptor regions.

Overall, interaction analysis showed the importance of certain amino acid residues in stabilizing the binding of PUFAs to BPH-related macromolecules. Hydrogen bonding and hydrophobic interactions play an important role in mediating these interactions. The identification of key residues involved in the binding of PUFAs provides valuable information for future structure-based drug design and the development of potential therapeutic agents for BPH.

DISCUSSION

N. salinicola has been studied for its fatty acid profile and its potential as a source of PUFAs [37]. The role of *N. salinicola* in the production of PUFAs is related to its ability to synthesize these compounds through specific pathways [38]. The fatty acid profile of *N. salinicola* is similar to that of other diatoms, with 14:0, 16:0, 16:1*n*-7, and 20:5*n*-3 (EPA), being the major fatty acids for polar and neutral lipids. The strong concentration of 16:1n-7 and its high enrichment supported its central role as a precursor of the C16 PUFA pathway. The C16 PUFA pathway is initiated by 16:1*n*-7, which is first desaturated in 16:2n-7 or 16:2*n*-4.

The position of the double bond in the fatty acid chain affects PUFA binding energy. PUFAs with a greater number of double bonds tend to exhibit more negative (lower) binding energies. This means that they have stronger interactions with the target macromolecule. This was because double bonds can enhance the flexibility and conformational adaptability of fatty acids, thereby allowing them to better fit into the binding pockets of the macromolecule.

The position of the first double bond from the omega end (methyl end) of the fatty acid chain also plays a role. Omega-3 PUFAs (e. g., α-linoleic acid, docosahexaenoic acid, and eicosapentaenoic acid) often exhibit more negative binding energies compared to omega-6 PUFAs (e. g., arachidonic acid and γ-linolenic acid). Omega-3 PUFAs are often linked to anti-inflammatory effects. Their extended carbon chain and higher number of double bonds may contribute to stronger interactions with the target, resulting in lower binding energies [39].

Monounsaturated fatty acids with a single, double bond (e. g., oleic acid and palmitoleic acid) tend to have moderate binding energies. While they have one double bond, their overall flexibility might be slightly limited compared to PUFAs with multiple double bonds. Elaidic acid, a *trans*-fatty acid, also demonstrates moderate binding energy. The *trans* configuration of the double bond could influence the interaction with the target, resulting in relatively moderate binding energy. The *cis*-10-heptadecenoic acid, with one double bond, displays moderate binding energy. The specific position of the double bond and its influence on interactions with the target contribute to this energy level.

In summary, the position and number of double bonds in PUFAs significantly impact their binding energy. Even though the binding energy of the omega-3 PUFAs was less than that of the native ligand,

PUFAs have advantages. Omega-3 PUFAs are a type of polyunsaturated fatty acid that is known for its anti-inflammatory effects and can be obtained from plant-based and marine sources. They have been shown to reduce the risk of cardiovascular disease, improve cognitive function, and improve depression [12]. PUFAs with higher numbers of double bonds and omega-3 configurations often exhibit stronger interactions with the target, resulting in more negative binding energies. The relationship between fatty acid structure and binding energy underscores the importance of understanding molecular interactions for potential therapeutic applications.

CONCLUSION

Molecular docking was used for *in silico* investigation of the inhibitory effect of benign prostate hyperplasia on STAT3 protein by 15 PUFAs from *N. salinicola.* PUFAs from *N. salinicola* were found to interact with the macromolecule STAT3 (PDB ID 6NJS). α-Linolenic acid and docosahexaenoic acid had substantial binding affinities for certain amino acid residues in the macromolecule's active site. Notably, α-linolenic acid exhibited strong hydrogen bonding and hydrophobic interactions, indicating its potential as a potent BPH inhibitor. These findings suggest that natural substances like PUFAs could be used as innovative and targeted treatment agents for BPH control. However, more research is needed to demonstrate the inhibitory effects of PUFAs on BPH and to understand the underlying molecular dynamics.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All the authors have equally contributed to the current study.

CONFLICT OF INTERESTS

All the authors declare no conflicts of interest.

REFERENCES

1. Speakman M, Kirby R, Doyle S, Ioannou C. Burden of male lower urinary tract symptoms (LUTS) suggestive of benign prostatic hyperplasia (BPH)-focus on the UK. BJU Int. 2015;115(4):508-19. doi: 10.1111/bju.12745, PMID 24656222.
2. Madersbacher S, Sampson N, Culig Z. Pathophysiology of benign prostatic hyperplasia and benign prostatic enlargement: a mini-review. Gerontology. 2019;65(5):458-64. doi: 10.1159/000496289, PMID 30943489.
3. Nunes Xavier CE, Angulo JC, Pulido R, Lopez JI. A critical insight into the clinical translation of PD-1/PD-L1 blockade therapy in clear cell renal cell carcinoma. Curr Urol Rep. 2019;20(1):1. doi: 10.1007/s11934-019-0866-8, PMID 30645700.
4. Mohammed M, Bayissa B, Getachew M, Adem F. Drug-related problems and determinants among elective surgical patients: a prospective observational study. SAGE Open Med. 2022;10:20503121221122438. doi: 10.1177/20503121221122438, PMID 36093421.
5. Rodrigues SP, Wever AM, Dankelman J, Jansen FW. Risk factors in patient safety: minimally invasive surgery versus conventional surgery. Surg Endosc. 2012;26(2):350-6. doi: 10.1007/s00464-011-1874-z, PMID 21898021.
6. Li Y, Shi B, Dong F, Zhu X, Liu B, Liu Y. Effects of inflammatory responses, apoptosis, and STAT3/NF-κB- and Nrf2-mediated oxidative stress on benign prostatic hyperplasia induced by a high-fat diet. Aging (Albany, NY). 2019;11(15):5570-8. doi: 10.18632/aging.102138, PMID 31412319.
7. Huang HF, Murphy TF, Shu P, Barton AB, Barton BE. Stable expression of constitutively activated STAT3 in benign prostatic epithelial cells changes their phenotype to that resembling malignant cells. Mol Cancer. 2005;4(1):2. doi: 10.1186/1476-4598-4-2, PMID 15647107.
8. Park WY, Song G, Park JY, Ahn KS, Kwak HJ, Park J. Ellagic acid improves benign prostate hyperplasia by regulating androgen signaling and STAT3. Cell Death Dis. 2022;13(6):554. doi: 10.1038/s41419-022-04995-3, PMID 35715415.
9. Lin J, Zhou J, Zhong X, Hong Z, Peng J. Inhibition of the signal transducer and activator of transcription 3 signaling pathway by qianliening capsules suppresses the growth and induces the apoptosis of human prostate cells. Mol Med Rep. 2015;11(3):2207-14. doi: 10.3892/mmr.2014.2946, PMID 25394909.
10. Lin J, Zhou J, Xu W, Zhong X, Hong Z, Peng J. Qianliening capsule treats benign prostatic hyperplasia via suppression of the EGF/STAT3 signaling pathway. Exp Ther Med. 2013;5(5):1293-300. doi: 10.3892/etm.2013.1008, PMID 23737867.
11. Duan J, Song Y, Zhang X, Wang C. Effect of ω-3 polyunsaturated fatty acids-derived bioactive lipids on metabolic disorders. Front Physiol. 2021;12:646491. doi: 10.3389/fphys.2021.646491, PMID 34113260.
12. Saini RK, Keum YS. Omega-3 and omega-6 polyunsaturated fatty acids: dietary sources, metabolism, and significance-a review. Life Sci. 2018;203:255-67. doi: 10.1016/j.lfs.2018.04.049, PMID 29715470.
13. Ventura S, Oliver Vl, White CW, Xie JH, Haynes JM, Exintaris B. Novel drug targets for the pharmacotherapy of benign prostatic hyperplasia (BPH). Br J Pharmacol. 2011;163(5):891-907. doi: 10.1111/j.1476-5381.2011.01332.x, PMID 21410684.
14. Sandhya S. Phylogenetic diversity, significance and future prospects of heterotrophic bacteria associated with marine microalgae; 2018.
15. Renugadevi K, Nachiyar CV, Nellore J, Sunkar S, Namasivayam SKR. Nutraceutical compounds from marine microalgae. Handbook of Nutraceuticals and Natural Products: Biological, Medicinal, and Nutritional Properties and Applications. 2022;1:245-55.
16. Kurnia D, Idar V, Angraeni VJ, Hendriani R, Asnawi A, Nurachman Z. Potential of navicula salinicola extract, a microalgae from maluku islands, as an anti-inflammatory agent using the human red blood cells (HRBC) method. Rasayan J Chem. 2022;15(2):1174-81. doi: 10.31788/RJC.2022.1526913.
17. Pekkoh J, Phinyo K, Thurakit T, Lomakool S, Duangjan K, Ruangrit K. Lipid profile, antioxidant and antihypertensive activity, and computational molecular docking of diatom fatty acids as ACE inhibitors. Antioxidants (Basel). 2022;11(2):186. doi: 10.3390/antiox11020186, PMID 35204069.
18. Jiang G, Sun C, Wang X, Mei J, Li C, Zhan H. Hepatoprotective mechanism of Silybum marianum on nonalcoholic fatty liver disease based on network pharmacology and experimental verification. Bioengineered. 2022;13(3):5216-35. doi: 10.1080/21655979.2022.2037374, PMID 35170400.
19. Pereira H, Barreira L, Figueiredo F, Custodio L, Vizetto Duarte C, Polo C. Polyunsaturated fatty acids of marine macroalgae: potential for nutritional and pharmaceutical applications. Mar Drugs. 2012;10(9):1920-35. doi: 10.3390/md10091920, PMID 23118712.
20. Gu Z, Suburu J, Chen H, Chen YQ. Mechanisms of omega-3 polyunsaturated fatty acids in prostate cancer prevention. BioMed Res Int. 2013;2013:824563. doi: 10.1155/2013/824563, PMID 23762859.
21. Veras ASC, Gomes RL, Almeida Tavares ME, Giometti IC, Cardoso APMM, da Costa Aguiar Alves B. Supplementation of polyunsaturated fatty acids (PUFAs) and aerobic exercise improve functioning, morphology, and redox balance in prostate obese rats. Sci Rep. 2021;11(1):6282. doi: 10.1038/s41598-021-85337-9, PMID 33737530.
22. Guertin MH, Robitaille K, Pelletier JF, Duchesne T, Julien P, Savard J. Effects of concentrated long-chain omega-3 polyunsaturated fatty acid supplementation before radical prostatectomy on prostate cancer proliferation, inflammation, and quality of life: study protocol for a phase IIb, randomized, double-blind, placebo-controlled trial. BMC Cancer. 2018;18(1):64. doi: 10.1186/s12885-017-3979-9, PMID 29321047.
23. Ghadian A, Rezaei M. Combination therapy with omega-3 fatty acids plus tamsulocin and finasteride in the treatment of men with lower urinary tract symptoms (LUTS) and benign prostatic hyperplasia (BPH). Inflammopharmacology. 2017;25(4):451-8. doi: 10.1007/s10787-017-0343-2, PMID 28391389.
24. Bercea CI, Cottrell GS, Tamagnini F, McNeish AJ. Omega-3 polyunsaturated fatty acids and hypertension: a review of vasodilatory mechanisms of docosahexaenoic acid and eicosapentaenoic acid. Br J Pharmacol. 2021;178(4):860-77. doi: 10.1111/bph.15336, PMID 33283269.

25. Alexander W. Prostate cancer risk and omega-3 fatty acid intake from fish oil: a closer look at media messages versus research findings. Pharm Ther. 2013;38:561.
26. Chen Z, Ge M. Discovering pathways in benign prostate hyperplasia: a functional genomics pilot study. Exp Ther Med. 2021;21(3):242. doi: 10.3892/etm.2021.9673, PMID 33603850.
27. Saah SA, Sakyi PO, Adu-Poku D, Boadi NO, Djan G, Amponsah D. Docking and molecular dynamics identify leads against 5 alpha-reductase 2 for benign prostate hyperplasia treatment. J Chem. 2023;2023:1-20. doi: 10.1155/2023/8880213.
28. Kim S, Chen J, Cheng T, Gindulyte A, He J, He S. PubChem 2019 update: improved access to chemical data. Nucleic Acids Res. 2019;47(D1):D1102-9. doi: 10.1093/nar/gky1033, PMID 30371825.
29. Bai L, Zhou H, Xu R, Zhao Y, Chinnaswamy K, McEachern D. A potent and selective small-molecule degrader of STAT3 achieves complete tumor regression *in vivo*. Cancer Cell. 2019;36(5):498-511.e17. doi: 10.1016/j.ccell.2019.10.002, PMID 31715132.
30. Balint M, Jeszenoi N, Horvath I, van der Spoel D, Hetenyi C. Systematic exploration of multiple drug binding sites. J Cheminform. 2017;9(1):65. doi: 10.1186/s13321-017-0255-6, PMID 29282592.
31. Jejurikar BL, Rohane SH. Drug designing in discovery studio. Asian J Res Chem. 2021;14(2):2-8.
32. Asnawi A, Nedja M, Febrina E, Purwaniati P. Prediction of a stable complex of compounds in the ethanol extract of celery leaves (Apium graveolens l.) Function as a vkorc1 antagonist. Trop J Nat Prod Res. 2023;7:2362-70.
33. Febrina E, Asnawi A, Abdulah R, Lestari K, Supratman U. Identification of flavonoids from Acalypha indica l. (euphorbiaceae) as caspase-3 activators using molecular docking and molecular dynamics. Int J Appl Pharm. 2022:162-6.
34. Febrina E, Alamhari RK, Abdulah R, Lestari K, Levita J, Supratman U. Molecular docking and molecular dynamics studies of Acalypha indica l. Phytochemical constituents with caspase-3. Int J App Pharm. 2021;13(4):210-5. doi: 10.22159/ijap.2021.v13s4.43861.
35. Peng T, Wonganan O, Zhang Z, Yu J, Xi R, Cao Y. A 2-benzylmalonate derivative as stat3 inhibitor suppresses tumor growth in hepatocellular carcinoma by upregulating β-TrCP e3 ubiquitin ligase. Int J Mol Sci. 2021;22(7):3354. doi: 10.3390/ijms22073354, PMID 33805945.
36. Du X, Li Y, Xia YL, Ai SM, Liang J, Sang P. Insights intoprotein–ligand interactions: mechanisms, models, and methods. Int J Mol Sci. 2016;17(2):144. doi: 10.3390/ijms17020144, PMID 26821017.
37. Etesami E, Saba F, Noroozi M, Amoozegar MA, Khaniki GB, Fazeli SAS. Caspian Sea's Navicula salinicola Hustedt 1939 and effect of the prolonged culture on its fatty acid profile. Int J Aquat Biol. 2017;5:268-74.
38. Remize M, Planchon F, Loh AN, Grand FL, Bideau A, Goic NL. Study of synthesis pathways of the essential polyunsaturated fatty acid 20:5n-3 in the diatom chaetoceros muelleri using ^{13}C-isotope labeling. Biomolecules. 2020;10(5):797. doi: 10.3390/biom10050797, PMID 32455747.
39. Oppedisano F, Macri R, Gliozzi M, Musolino V, Carresi C, Maiuolo J. The anti-inflammatory and antioxidant properties of n-3 PUFAs: their role in cardiovascular protection. Biomedicines. 2020;8(9):306. doi: 10.3390/biomedicines8090306, PMID 32854210.