Pharmacogn. Res., 2024; 16(3):626-637.
https://www.phcogres.com

Original Article

Network Pharmacology and Molecular Docking Approaches of Astaxanthin (ATX) against Atherosclerosis

Anis Zuhaida Mamnun Barizi[1], Ain Nabila Syahira Shamsol Azman[1], Muhamad Fikri Shazlan Saad[1], Muhammad Nazrul Hakim Abdullah[2], Vuanghao Lim[3], Yoke Keong Yong[1],*

[1]Department of Human Anatomy, Faculty of Medicine and Health Sciences, University Putra Malaysia, Serdang, Selangor, MALAYSIA.
[2]Department of Biomedical Science, Faculty of Medicine and Health Sciences, University Putra Malaysia, Serdang, Selangor, MALAYSIA.
[3]Advanced Medical and Dental Institute, University Sains Malaysia, Bertam, Penang, MALAYSIA.

ABSTRACT

Background: Atherosclerosis is an inflammatory disease well known as the leading cause of Cardiovascular Diseases (CVDs). Astaxanthin (ATX) is a reddish pigment that belongs to the family of xanthophylls, which are oxygenated derivatives of carotenoids. **Aim:** In this study, we aimed to investigate the targets and mechanisms involved in treating atherosclerosis using network pharmacology and molecular approaches. **Materials and Methods:** The genes targeted by ATX were predicted using Swiss Target Prediction, BATMAN-TCM, and Super-Pred databases, while genes associated with atherosclerosis were retrieved from DigSee, GAD, GeneCards, and OMIM databases. The interactions between ATX and atherosclerosis genes were identified through protein–protein interaction analysis, Gene Ontology (GO) enrichment analysis, and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis. **Results:** The results revealed a total of 240 ATX-related genes and 4,977 atherosclerosis-related genes, with 172 overlapping genes identified. Six core genes were obtained: SRC, AKT1, MAPK3, HDAC1, PIK3R1, and RXRA. These results were further validated through the molecular docking approach, where all six core targets exhibit low binding energy, suggesting strong binding affinity, with PIK3R1 having the best binding affinity among them. **Conclusion:** Our study provides novel insights into the potential application of ATX in the management of atherosclerosis.

Keywords: Atherosclerosis, Astaxanthin, Network Pharmacology, Carotenoids, GO, KEGG.

Correspondence:
Dr. Yong Yoke Keong
Associate Professor, Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, MALAYSIA.
Email: yoke_keong@upm.edu.my

Received: 12-01-2024;
Revised: 10-03-2024;
Accepted: 04-04-2024.

INTRODUCTION

Cardiovascular Disease (CVD) is the chief of death worldwide. According to World Health Organization (WHO) in 2019, an estimated 32% of global deaths were attributable to CVDs. Atherosclerosis is an inflammatory disease and is recognized to be the main cause of CVDs. It is described by an accumulation of plaque, narrowing, and hardening of coronary artery walls which later can manifest myocardial infarction, angina pectoris, and sudden death.[1] Presently, the prevalent therapy to lessen the symptoms of atherosclerosis is therapeutic treatment with medications, angioplasty, or coronary artery bypass surgery, however, these treatments may have some disadvantages.[2] Even though there have been considerable therapeutic advancements in recent years, it is still crucial to increase clinical medicine resources, particularly from natural resources, such as plants.

Carotenoids are pigments found in plants, algae, and certain microorganisms. Due to the presence of carotenoids, carrots, tomatoes, and peppers, among other fruits and vegetables, exhibit yellow, orange, or red colors.[3] On top of that, carotenoids may also be found in spinach and kale, although their color is masked by chlorophyll. In the 1970s and 1980s, the antioxidant potential of carotenoids was first revealed.[4] Since then, scientists have discovered over 600 unique carotenoid compounds. The majority of these substances occur naturally in fruits and vegetables. It is believed that these chemicals may prevent a variety of illnesses, including cancer, cardiovascular disease, and neurological disorders.[4] Carotenoids may also play a role in the prevention and treatment of hypertension, diabetes, cataracts, age-related macular degeneration, and atherosclerosis, according to accumulating data.[3] Although practically all carotenoids possess antioxidant capabilities, not all have the same level of antioxidant activity. Studies have revealed, for instance, that ATX provides much more protection than alpha-tocopherol or beta-carotene.[5]

Astaxanthin (ATX) is a reddish pigment that belongs to the family of xanthophylls, the oxygenated derivatives of carotenoids.[6] ATX is often found in aquatic animals such as salmon, trout, lobster, fish eggs, red seabream, and shrimp, as well as in birds such as

DOI: 10.5530/pres.16.3.74

flamingos and quails.[7] ATX has been reported to possess a variety of pharmacological activities, including anti-inflammatory and antioxidative properties, which are closely related to each other and may be beneficial for cardiovascular function.[8,9] Therefore, ATX may have a promising therapeutic effect on atherosclerosis.

Network pharmacology is a field of study that integrates systems biology, network analysis, and pharmacology to understand the complex interactions between drugs and biological systems at a global level.[10] It aims to identify the key molecular targets and pathways underlying the therapeutic effects and adverse reactions of drugs, as well as to discover new drug targets and combinations for the treatment of complex diseases. Network pharmacology considers drugs and disease processes as complex networks of molecular interactions, where nodes represent genes, proteins, and other biomolecules, and edges represent physical or functional associations between them. By analyzing these networks using computational and experimental methods, network pharmacology can provide a holistic and unbiased view of drug action and disease mechanisms, which can facilitate drug discovery and development.

Molecular docking is a structure-based approach to drug design that mimics molecular interactions to forecast the binding mechanism and affinity between ligands and receptors. This approach is practical for researchers to acquire, synthesize, and carry out subsequent pharmacological studies, while also improving efficiency and lowering research costs.[11] Docking was initially introduced in the mid-1970s and has since been shown to be a valuable method for medication development and discovery as well as for understanding how chemical compounds interact with their molecular targets.[12] Furthermore, docked compounds are ranked according to the binding affinity of ligand-receptor complexes using molecular docking algorithms, which carry out quantitative predictions of binding energetics.[13] Thus, we aim to explore the anti-atherosclerotic effect of ATX with the aid of network pharmacology and molecular docking approaches by predicting the main targets and possible mechanisms as well as the binding affinity.

MATERIALS AND METHODS

Screening of potential targets for ATX

The potential targets related to ATX were conducted by importing the Simplified Molecular Input Line Entry Specification (SMILES) or International Chemical Identifier (InChI) information into Swiss Target Prediction (http://www.swisstargetprediction.ch/, accessed on 14 July 2022), Bioinformatics Analysis Tool for Molecular Mechanism of Traditional Chinese Medicine (BATMAN-TCM, http://bionet.ncpsb.org.cn/batman-tcm/, accessed on 14 July 2022), and Super-PRED (https://prediction.charite.de/index.php, accessed on 14 July 2022). All three web tools provide a huge database for the prediction of compound targets. In BATMAN-TCM, the predicted candidate targets (including known targets) with scores not smaller than Score cut-off = 20 for each ingredient were presented. In addition, The Comparative Toxicogenomic Database (CTD) (https://ctdbase.org/) was also used to gain more information of interactions of ATX and target genes.

Screening potential target of atherosclerosis

To search for atherosclerosis-related genes, four resources that provide a very informative database of bioinformatic web tools were retrieved. DigSee is a text-mining search engine that shows how "genes" cause "diseases" via "biological processes" (http://210.107.182.61/geneSearch/, accessed on 15 July 2022). The Genetic Association Database (GAD) resource included all published genetic association studies where the data and metadata from each study have been standardized (https://maayanlab.cloud/Harmonizome/, accessed on 15 July 2022). Gene set for atherosclerosis disease from the GAD Gene-Disease Association will be selected for the current study. Searchable and integrated database GeneCards offers extensive and user-friendly information on all annotated and predicted human genes. Gene-centric data from 150 online sources, including genomic, transcriptomic, proteomic, genetic, clinical, and functional information, is automatically integrated into the knowledgebase (https://www.genecards.org/, accessed on 15 July 2022). OMIM is a comprehensive, authoritative collection of human genes and genetic traits that is publicly accessible and updated daily. The full-text referenced overviews in OMIM offer information on all known Mendelian illnesses and over 16,000 genes. OMIM focuses on the link between phenotype and genetics. It is updated frequently, and the posts include numerous connections to other genetics information (https://www.omim.org/, accessed on 15 July 2022). Protein targets related to atherosclerosis were discovered by merging the genes acquired from the four databases as mentioned previously after eliminating duplicates.

Construction of Venn Diagram

The collected targets for both ATX and atherosclerosis from the databases mentioned previously were imported into the Interactive Venn (http://www.interactivenn.net/, accessed on 21 July 2022) to identify the common potential therapeutic targets between ATX and atherosclerosis-related targets. The tool's Venn diagram depicts the points where possible target genes for drugs and diseases overlap.

Protein-Protein Interaction (PPI) data analysis

A Protein–Protein Interaction (PPI) network was established by inserting the target genes of ATX against atherosclerosis into The Search Tool for the Retrieval of Interacting Genes, STRING database, version 11.0 to gain a deeper comprehension of the protein interactions.[14] The "*Homo sapiens*" organism was used for the PPI networks, with the maximum confidence (0.900)

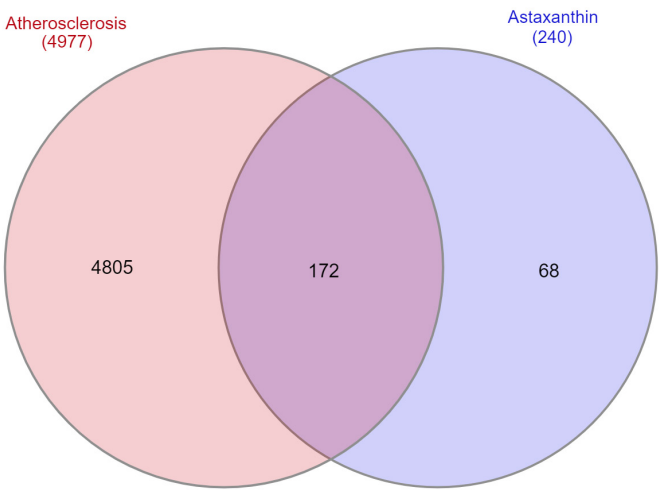


Figure 1: The relation of potential target genes via Venn diagram between ATX against atherosclerosis.

for the needed interaction score, and the unconnected node was removed. Following that, Molecular Complex Detection (MCODE) topology analysis was performed to determine the key subnetworks and genes. The parameters of MCODE were set as follows: degree cutoff = 2, node score cutoff = 0.2, K-score = 2, and Max depth = 100. Moreover, the CytoHubba plug-in from the Cytoscape software was used to further analyse the potential components and targets based on degree, closeness and betweenness.

Construction of PPI network

The data collected from the PPI analysis were imported into Cytoscape software to construct the interaction networks including: (i) PPI network of the compound target was built by connecting the ATX and other human proteins that interacted with them, and (ii) PPI network of ATX target against atherosclerosis was built by intersecting the two targets obtained from the Venn diagram.

Enrichment analysis

The hub genes were then imported into The Database for Annotation, Visualization, and Integrated Discovery, DAVID, version 6.8 (https://david.ncifcrf.gov/home.jsp, accessed on 1st July 2022) to run the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis where the species was set to Homo sapiens.[15] The purpose of the enrichment analysis that was carried out was to unearth the underlying mechanism by which the ATX combats atherosclerosis through the Biological Processes (BP), Cellular Components (CC), Molecular Function (MF), and critical signaling pathways.

Molecular docking

Common targets acquired from network topology analysis were performed in docking analysis as described in.[16] The crystal structure of six common targets (SRC, AKT1, MAPK3, HDAC1, PIK3R1 and RXRA) were retrieved from Protein Data Bank (https://www.rcsb.org/, retrieved on 17 February 2024). The impurities from the macromolecule such as unwanted binding, ligand molecules, and water molecules were removed by using Biovia Discovery Studio 2021 (https://discover.3ds.com/discovery-studio-visualizer, assessed on 17 February 2024). Polar hydrogen, Kollman charges, and other adjustments were introduced to the protein to enhance the interactions. These proteins underwent docking research against ATX, for which the PubChem database provided the 3D structure (https://pubchem.ncbi.nlm.nih.gov/, retrieved on 17 February 2024) by using AutoDockTools 4.2. (http://autodock.scripps.edu/, assessed on 18 February 2024). The graphic representation of the interactions was visualized by Biovia Discovery Studio 2021.

RESULTS

Potential targets of ATX

Potential targets of ATX were obtained from three different databases, including BATMAN (42 potential targets), SuperPred (112 potential targets), and SwissPredict (108 potential targets). After removing the duplication among these three databases, a total of 240 potential targets of ATX were collected (Supplementary Table S1).

Potential targets of atherosclerosis

Four databases were used to retrieve the potential target for the atherosclerosis disease, including Genecards (4739 potential targets), GAD (354 potential targets), OMIM (344 potential targets), and DigSee (1099 potential targets). After removing the redundant or duplicate potential targets, a total of 4977 atherosclerosis-related targets were identified (Supplementary Tables S2 to S5).

Identification of candidate targets of ATX for atherosclerosis treatment

Both atherosclerosis-related and ATX-related potential targets were correlated, and a total of 172 targets (Supplementary Table S6) were identified as the potential targets of ATX against atherosclerosis (Figure 1).

PPI network analysis

The intersection potential targets between ATX against atherosclerosis were imported into the STRING database to obtain the Protein-Protein Interaction (PPI) data analysis by setting the interaction score to the highest confidence (0.900). According to the analysis, the network involved 118 nodes and 413 edges, with an average node degree of 7 and PPI enrichment

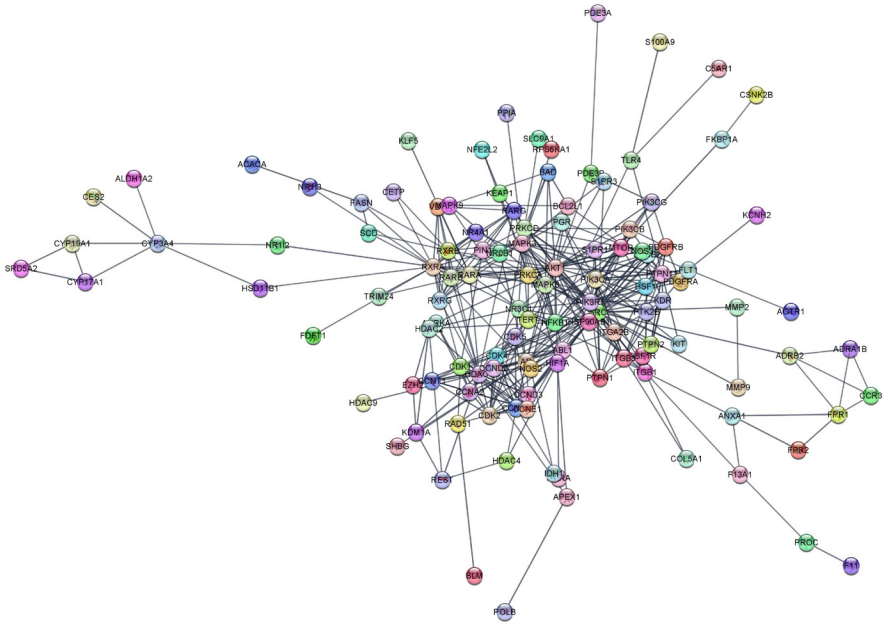


Figure 2: Protein-protein interaction (PPI) network of ATX targets against atherosclerosis.

p-value < 1.0e-16 (Figure 2). There was a total of six clusters detected via MCODE (Figure 3). Cluster 1 consisted of 23 nodes and 202 edges with two core nodes: MTOR and HSP90AB1; Cluster 2 consisted of 27 nodes and 164 edges with a core node of AKT1; Cluster 3 consisted of 6 nodes and 12 edges with two core nodes, PROC and F10; Cluster 4 consisted of 7 nodes and 9 edges with core node of RXRA; Cluster 5 and 6 consisted of 3 nodes and 3 edges, respectively. However, there is no core node available for Cluster 5 and 6 due to less than 4 nodes for further analysis. To analyze the complex PPI network, network topology analysis was further determined according to the three parameters including the degree of a node, closeness centrality, and betweenness centrality. Overall, six genes were determined as possessing high values of degree, closeness and betweenness, including SRC, AKT1, MAPK3, HDAC1, PIK3R1 and RXRA.

Construction of protein interaction network

After getting all probable intersecting targets between ATX and atherosclerosis, the data was exported into the STRING database to determine the link between the targets. The generated files were then processed to the Cytoscape programme for Protein-Protein Interaction (PPI) complex diagrams in a manner that could be used to illustrate the association between ATX and atherosclerosis intersection genes (Figure 4).

Analysis via gene ontology for target pathway

The DAVID database (https://david.ncifcrf.gov/home.jsp) was used to investigate GO function enrichment and KEGG pathway enrichment for the aforementioned 172 common targets (Figure 2). Together, Biological Processes (BP), Cellular Components (CC), and Molecular Functions (MF) explain the roles of gene products in GO analysis. As shown in Tables 1-3, the GO analysis of ATX in treating atherosclerosis mainly involved in inflammatory response, positive regulation of cell proliferation, response to xenobiotic stimulus, positive regulation of smooth muscle cell proliferation and apoptotic process (biological process); cytoplasm, nucleoplasm, receptor complex, cyclin-dependent protein kinase holoenzyme complex and plasma membrane (cellular component), while RNA polymerase II transcription factor activity, ligand-activated sequence-specific DNA binding, enzyme binding, protein binding, sequence-specific DNA binding and protein kinase binding in molecular function. This indicates that ATX may have a role in the therapy of atherosclerosis by acting on different targets via diverse signalling pathways, as shown by an enrichment study. In addition, it offers a valuable reference for future searches for critical core targets and chemicals.

KEGG enrichment analysis

KEGG enrichment analysis was performed using DAVID to unravel the underlying mechanism of ATX in treating atherosclerosis. There was total of 120 KEGG pathways were filtered with applicable thresholds *p* values < 0.01, 20 of which with the lowest *p* values were selected for further analysis, including PI3K-Akt signaling pathway, lipid and atherosclerosis, neutrophil extracellular trap formation, fluid shear stress and atherosclerosis, thyroid hormone signaling pathway, endocrine resistance and AGE-RAGE signaling pathway in diabetic complications (Figure 5). Overall, the enrichment analysis revealed that most of the therapeutic targets were associated with cancer, infection, and inflammation which suggests that ATX may possess an anti-atherogenic effect via anti-inflammation processes.

Table 1: GO biological process.

Description	Count in gene set	False Discovery Rate (FDR)
Inflammatory response	31	7.54E-16
Positive regulation of cell proliferation	33	1.72E-14
Response to xenobiotic stimulus	23	1.87E-13
Positive regulation of smooth muscle cell proliferation	15	6.76E-13
Positive regulation of the apoptotic process	24	9.02E-12
Protein phosphorylation	28	1.72E-11
Positive regulation of gene expression	28	4.13E-11
Response to drug	22	4.28E-11
Positive regulation of transcription from RNA polymerase II promoter	40	2.35E-10
Negative regulation of the apoptotic process	27	2.53E-10

Table 2: GO cellular component.

Description	Count in gene set	False Discovery Rate (FDR)
Cytoplasm	99	1.92E-14
Nucleoplasm	73	6.80E-10
Receptor complex	16	4.41E-08
Cyclin-dependent protein kinase holoenzyme complex	9	1.04E-07
Plasma membrane	80	3.56E-07
Perinuclear region of cytoplasm	25	1.10E-06
Chromatin	29	2.14E-06
Cytosol	79	3.04E-06
Macromolecular complex	23	3.04E-06
Integral component of plasma membrane	32	2.04E-05

Table 3: GO molecular function.

Description	Count in gene set	False Discovery Rate (FDR)
RNA polymerase II transcription factor activity, ligand-activated sequence-specific DNA binding	16	3.97E-16
Enzyme binding	23	2.15E-09
Protein binding	153	2.59E-09
Sequence-specific DNA binding	20	2.22E-08
Protein kinase binding	24	2.57E-08
Steroid binding	8	6.68E-07
Protein kinase activity	19	8.26E-07
Identical protein binding	41	1.28E-06
Transcription factor binding	14	3.50E-06
Transmembrane receptor protein tyrosine kinase activity	11	5.39E-06

Table 4: Target docking details and corresponding scores.

Compound	Targets	PDB ID	Binding Energy (kcal/mol)
ATX	PIK3R1	5GJI	-11.15
	RXRA	6JNO	-8.91
	AKT1	6CCY	-8.53
	MAPK3	4QTB	-8.10
	SRC	3G5D	-7.34
	HDAC1	5ICN	-6.44

Molecular docking

The scores of molecular docking analysis are as follows; PIK3R1 (-11.15 kcal/mol), RXRA (-8.91 kcal/mol), AKT1 (-8.53 kcal/mol), MAPK3 (-8.10 kcal/mol), SRC (-7.34 kcal/mol) and HDAC1 (-6.44 kcal/mol) also displayed in Table 4. In accordance with the results, all targets possess binding energies which are regarded as excellent binding interaction, with PIK3R1 being particularly the lowest among them. This indicates a tendency for PIK3R1 and ATX to bind and have an influence on one another. Moreover, the results demonstrate that ATX forms two hydrogen bonds with Ser-361 and Arg-358 in PIK3R1 while the remaining interactions of other targets are depicted in Figure 6 (i-vi).

DISCUSSION

Salmon, shrimp, and lobster are all good sources of the carotenoid pigment known as ATX, which may be found in these foods. It is well recognised for its significant antioxidant capabilities that it has, and it has been the subject of much research for the possible health advantages that it may provide, including its involvement in the development of cardiovascular disorders. Inflammation and oxidative stress are a common factor in the development of cardiovascular disorders including heart attacks and strokes. It

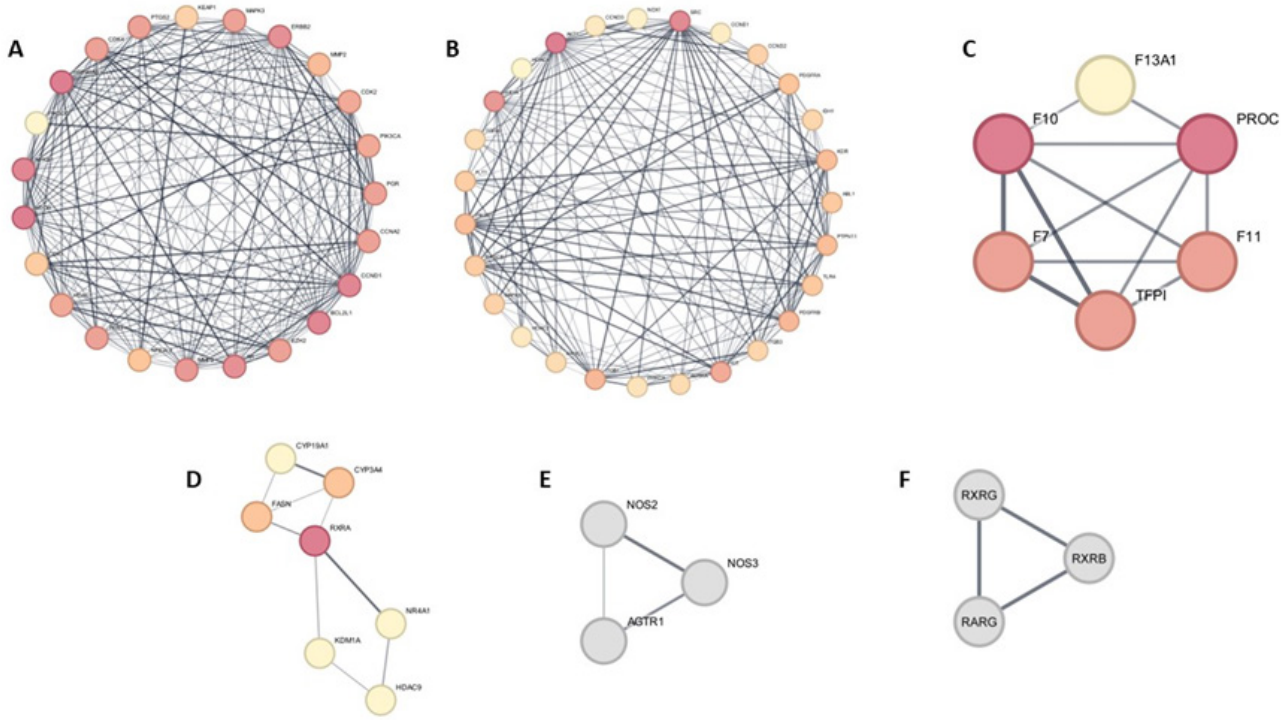


Figure 3: MCODE analysis of PPI network of 118 potential anti-atherosclerosis key targets. (A) Cluster 1 (B) Cluster 2 (C) Cluster 3 (D) Cluster 4 (E) Cluster 5 and (F) Cluster 6. The color of each node changes from red (highest) to yellow (lowest) as its degree decreases.

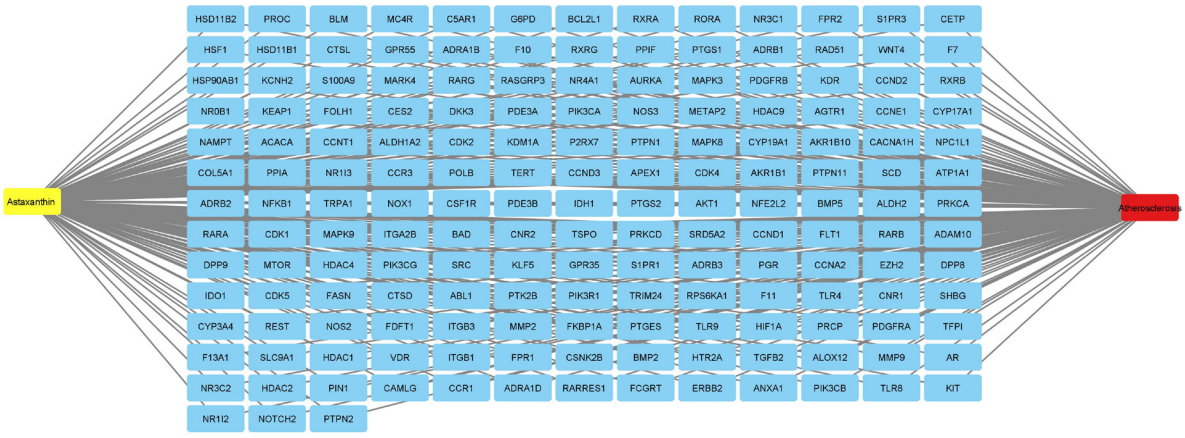


Figure 4: Network of intersection targets between ATX and atherosclerosis.

has been shown that ATX exhibited a number of pharmacological activities including antioxidant and anti-inflammatory activities.[8-9,17-19] Studies also suggested that ATX possessed cardioprotective effects, such as suppression of LDL oxidation which is one of the main contributors to atherosclerosis,[20] and reversed cholesterol transport.[21] While a few studies have established the therapeutic benefits of ATX against atherosclerosis and related disorders, the therapeutic mechanisms have not yet been thoroughly explained. As a result of this, the current research was carried out to investigate the pharmacological mechanisms of ATX in relation to atherosclerosis by utilizing network pharmacology analysis.

Through network pharmacology analysis, 172 common genes were discovered between ATX and atherosclerosis. 118 nodes and 413 edges have been identified through the PPI network,

demonstrating an intricated and interlinked network of connections. Among all the genes, there were six genes were determined as possessed with high values of degree, closeness, and betweenness, including SRC, AKT1, MAPK3, HDAC1, PIK3R1, and RXRA. The six aforementioned were believed to be potential candidates for ATX in the management of atherosclerosis. Following are discussions in turn of each of the six genes.

Src-family kinases mediate macrophage functions, comprising foam cell formation, proinflammatory cytokine expression, efferocytosis, and lesion development in a mouse model of atherosclerosis.[22] By suppressing NF-κB, ATX has been demonstrated to reduce the secretion of inflammatory mediators such as TNF and ILs in activated M1 macrophages. In specific, ATX may be beneficial for preserving Src Homology Phosphatase (SHP) 2, a negative regulator of NF-κB activity.[23] Besides, AKT1 is one of three serine/threonine protein kinases (AKT1, AKT2, and AKT3) that control an array of processes, such as metabolism, proliferation, cell survival, growth, and angiogenesis.[24] According to research, the PI3K/Akt signaling pathway is critical in the onset and progression of atherosclerosis. After PI3K phosphorylates Akt, it can effectively act on downstream target proteins such as B-cell lymphoma-2 gene-associated promoter (Bad), cysteine-aspartic protease (caspase-9), glycogen synthase kinase-3 (GSK-3), mammalian target of rapamycin (mTOR), and endothelial nitric oxide synthase (eNOS).[25] Through PI3K/Akt, ATX may reduce the activity of mTOR, GSK-3, HIF-1, Bcl-2, Bad, FoxO, and other associated mediators.[26] Thus, inflammation, atherosclerosis, and angiogenesis can all be significantly inhibited by highly activating the PIK3R1/Akt pathway.[27]

Mitogen-Activated Protein Kinase 3 (MAPK3), also known as Extracellular Signal-Regulated Kinase 1 (ERK1), is a crucial cell signaling molecule in the ERK/MAPK pathway. MAPK3 acts in multiple critical signaling transduction pathways in the development of atherosclerosis, including the Toll-like receptor, TGF-, PI3K-Akt, MAPK, and mTOR signaling pathways.[28] Moreover, it has been demonstrated that ATX not only reduced oxidative stress[29] and histopathological damage in rodents with hepatic lesions caused by ischemia/reperfusion,[30] but it also had a significant anti-inflammatory effect, inhibiting the release of inflammatory cytokines via the Mitogen-Activated Protein Kinase (MAPK) pathway.[31] In addition, ATX also proved to inhibits the reaction of bisphenol A-induced increased phosphorylation of MAPK3 protein in human dermal fibroblasts.[32] Taken together, ATX might against the atherosclerosis by acting on MAPK3 signaling pathway.

HDAC1 is acknowledged for modulating the cell cycle, angiogenesis, apoptosis, differentiation, and metastasis.[20] It was discovered that miR-410 may target HDAC1, while HDAC1 could target transcription factor KLF5, causing IKBα expression to rise and NF-kB to be suppressed in atherosclerosis. In addition, in HUVECs designed to model atherosclerosis, suppressing miR-410 or overexpressing HDAC1 enhanced cell survival and reduced apoptosis and inflammatory response. By increasing IKBα levels through KLF5 and suppressing NF-kB, blocking miR-410 promotes the production of HDAC1 and delays the onset of atherosclerosis.[33] According to Venkidasamy and his team,[34] ATX successfully inhibited the upregulation of HDAC1 expression induced by Lipopolysaccharide (LPS) in mammary epithelial cells. This data may provide evidence that ATX could

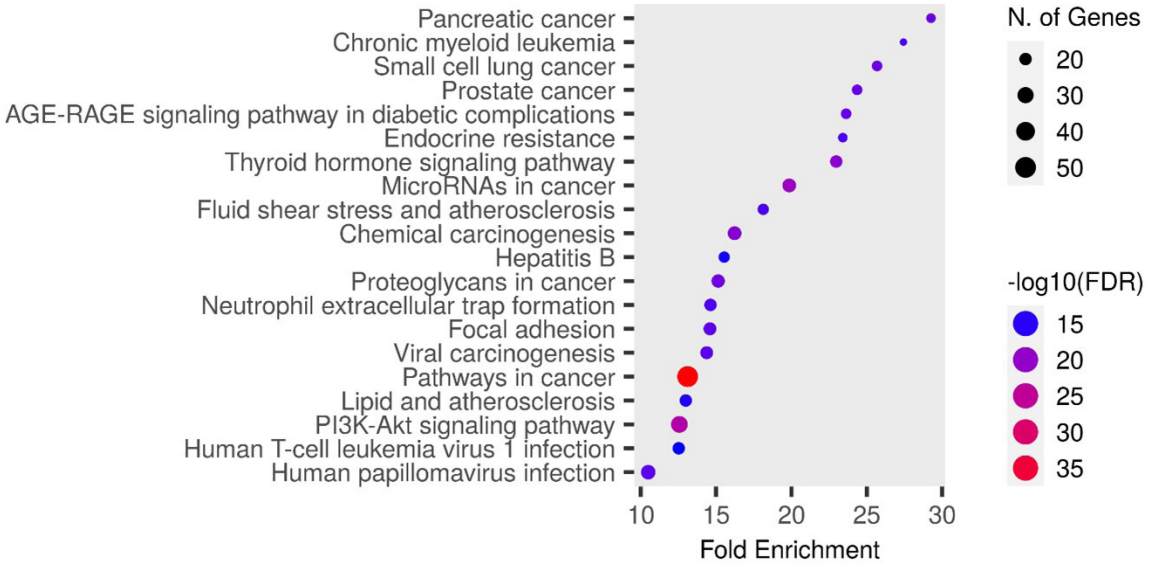
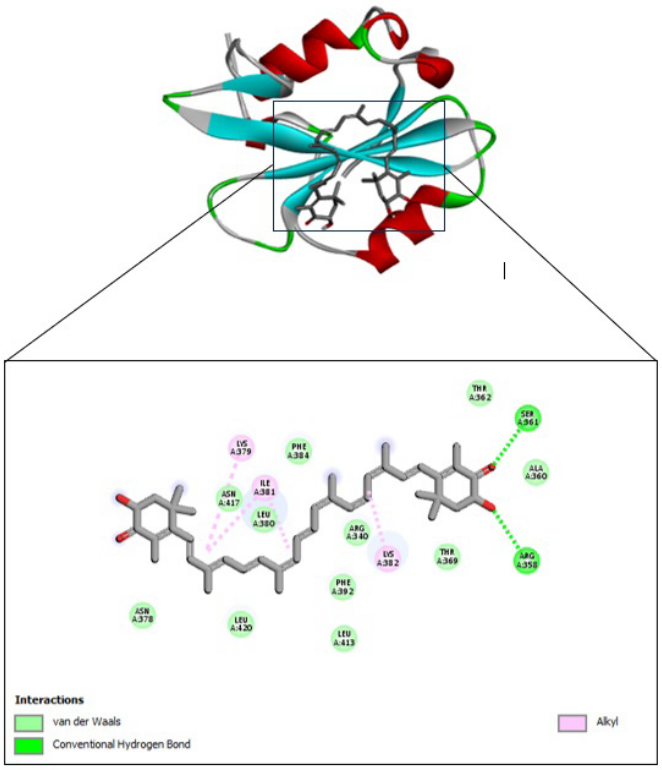
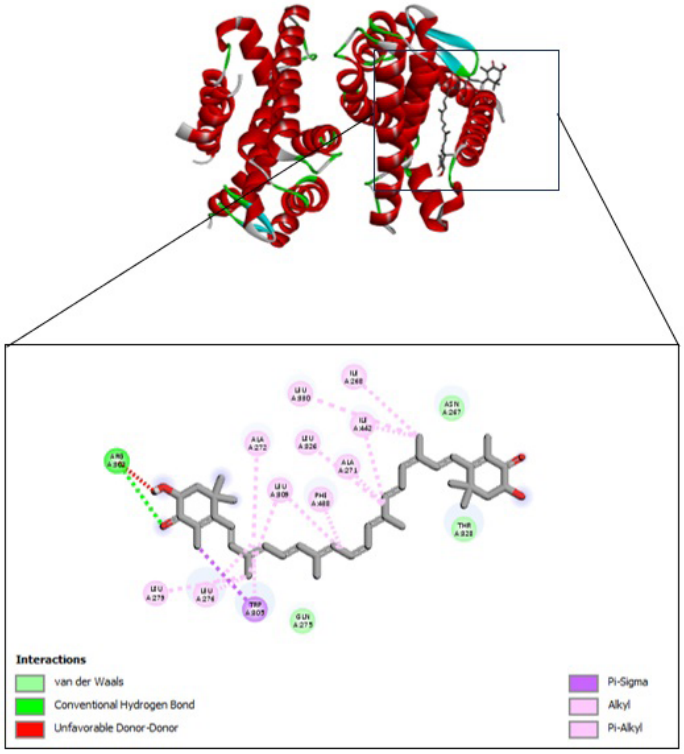


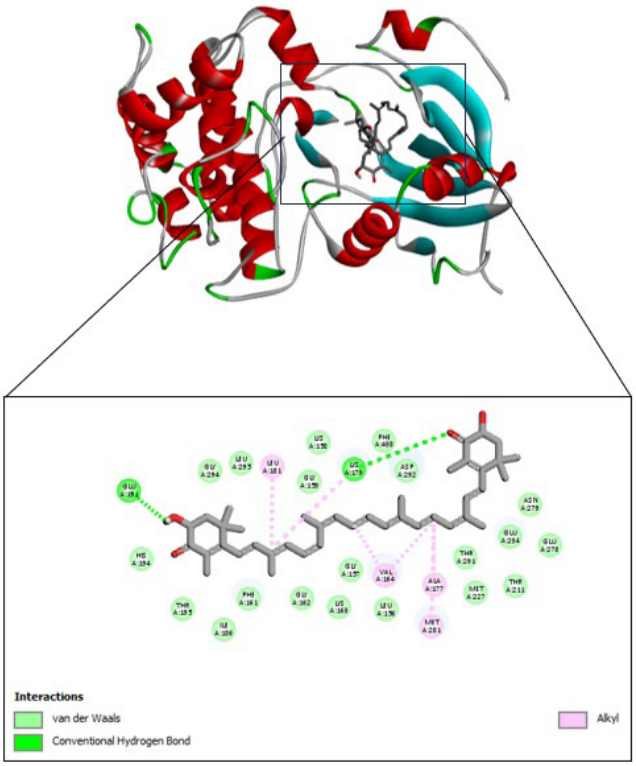
Figure 5: KEGG enrichment analysis for the 172 shared ATX compound targets against atherosclerosis-related targets. The Y-axis represents a significant KEGG pathway, and the X-axis represents the ratio of enriched targets in a pathway to all common targets. The size of the nodes shows the count of targets, and the gradient of colour represents the -log10 False Discovery Rate (FDR) where FDR < 0.01.



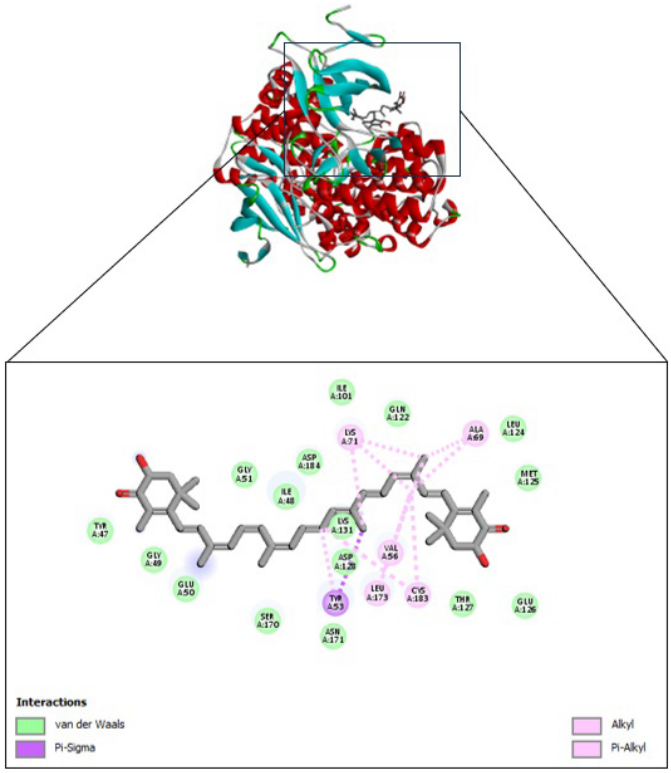
(i) PIK3R1 (PDB ID: 5GJI).



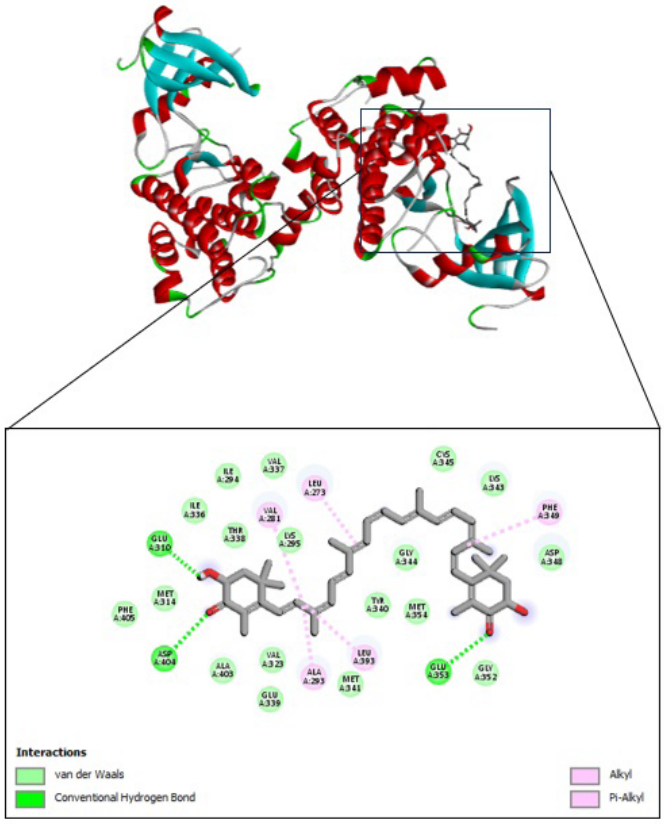
(ii) RXRA (PDB ID: 6JNO).



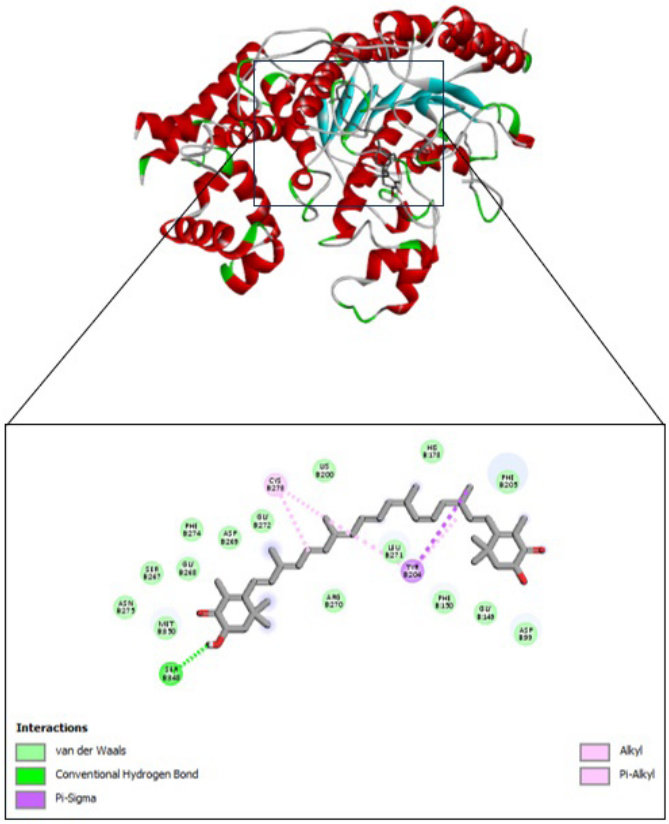
(iii) AKT1 (PDB ID: 6CCY).



(iv) MAPK3 (PDB ID: 4QTB).



(v) SRC (PDB ID: 3G5D).



(vi) HDAC1 (PDB ID: 5ICN).

Figure 6: The graphic representation of molecular docking results and corresponding indicator of interactions. (i) PIK3R1 (PDB ID: 5GJI), (ii) RXRA (PDB ID: 6JNO), (iii) AKT1 (PDB ID: 6CCY), (iv) MAPK3 (PDB ID: 4QTB), (v) SRC (PDB ID: 3G5D), (vi) HDAC1 (PDB ID: 5ICN).

suppress the process of atherosclerosis through the suppression of the HDAC1 gene.

The Retinoid X Receptor (RXR) is an important member of the steroid/thyroid hormone superfamily of Nuclear Receptors (NRs) that primarily serve as transcription factors in development, cell differentiation, metabolism, and cell death.[35] RXRs are crucial macrophage mediators and are involved in inflammatory and metabolic diseases. RXRa and RXRb are produced by macrophages and by regulating the uptake of apoptotic cells, b-amyloid clearance, inflammation, pathogen elimination, cholesterol transport, and lipid management, RXRs contribute to the integration of macrophage immunological functions and lipid metabolism. Atherosclerosis, neurodegeneration, autoimmunity, and immune system problems develop by abnormalities in these RXR-mediated processes.[36] There is, however, insufficient study on the relationship between ATX and atherosclerosis that is mediated through RXRA and PIK3R1.

To reveal the occurrence and progression of atherosclerosis, we sought out a few crucial GO and KEGG pathways with lower FDR and *p*-values respectively. In this study, enrichment analysis of GO and KEGG pathways was conducted on 172 common genes. In the results of the analysis, the five core targets were mainly involved in biological processes, including inflammatory response, positive regulation of cell proliferation, response to xenobiotic stimulus, positive regulation of smooth muscle cell proliferation, and apoptotic process. These biological processes are crucial for the progression of atherosclerosis. In addition, the five core targets also affected molecular functions (RNA polymerase II transcription factor activity, ligand-activated sequence-specific DNA binding, enzyme binding, protein binding, sequence-specific DNA binding, and protein kinase binding) and cellular components (cytoplasm, nucleoplasm, receptor complex, cyclin-dependent protein kinase holoenzyme complex, and plasma membrane) in relation to atherosclerosis.

Several core targets were chosen from KEGG pathway analysis, based on their significant association with atherosclerosis. PI3K-Akt signaling pathway as mentioned above is crucial for the onset and progression of AS whereas, for lipid and atherosclerosis, it is widely acknowledged that lipids play a significant role in the pathophysiology of atherosclerosis, from LDL uptake by monocytes and macrophages and accumulation in the arterial intima to their contribution to inflammation.[37] Next, Neutrophil Extracellular Trap (NET) formation is also involved in atherosclerosis. Myeloperoxidase from NETs may trigger macrophages to oxidize Low-Density Lipoprotein

(LDL) to ox-LDL and become the foam cell.[38] Additionally, although atherosclerosis is a complex multifactorial process, the pathogenesis of atherosclerotic plaque is significantly affected by fluid shear stress, particularly when blood flow is disturbed and/or nonlaminar especially when flow rates are disrupted by low or oscillatory shear stress.[39] Furthermore, thyroid hormone exerts direct anti-atherosclerotic properties such as dilatation of blood vessels, the formation of vasodilatory molecules, and suppression of the expression and signal transduction of the angiotensin II receptor.[40] Finally, the significance of Advanced Glycation End-Products (AGEs) in atherosclerosis, particularly in the presence of diabetes, and their association with the receptor RAGE are increasingly being supported by scientific research. Therefore, through our analysis, we speculate that ATX may regulate these key targets and pathways and result in an even greater improvement in atherosclerosis.

Moreover, molecular docking was employed to validate and reinforce the results of network pharmacology. The degree of affinities between a macromolecule and a ligand was ascertained using the binding energy. Lower binding energy is associated with a stronger affinity and more stable conformation where less than -5 kcal/mol signifies good binding interaction. The fact that all targets possess good binding interactions following the acquisition of top hub genes from network pharmacology demonstrates the accuracy and consistency of this approach. Therefore, the incorporation of network pharmacology and molecular docking provides a perspective to initiate an investigation regarding potential therapeutic strategies for certain diseases—in this case, ATX's effect on atherosclerosis.

Network pharmacology and molecular docking approaches were used to determine the potential targets and significant pathways as well as the binding affinity of ATX against atherosclerosis. However, the results provide only a preliminary theoretical basis for further experimental inquiry. Although the pharmacological mechanism of the ATX for the regulation of atherosclerosis is based on computational methods, it still has to be validated by experimental methods to further explore the potential mechanisms of ATX for atherosclerosis at molecular and cellular levels. Besides, due to inadequate database information, several key targets and active molecules may be overlooked in this investigation. Furthermore, multiple signaling pathways of ATX acting on atherosclerosis were predicted using the network pharmacology strategy, but the role of each pathway has not been fully understood.

CONCLUSION

In conclusion, ATX may have significant effects on the regulation of atherosclerosis through their effects on key targets namely, SRC, AKT1, MAPK3, HDAC1, PIK3R1, and RXRA as well as key pathways including PI3K-Akt signaling pathway, lipid and atherosclerosis, neutrophil extracellular trap formation, fluid shear stress and atherosclerosis, thyroid hormone signaling pathway and AGE-RAGE signaling pathway in diabetic complications. Besides, PIK3R1 exhibited the highest binding affinity towards ATX, followed by other key targets.

ABBREVIATIONS

AGE: Advanced Glycation End-products; **AGE-RAGE:** Advanced Glycation End Products-Receptor for Advanced Glycation End Products; **AKT:** Protein Kinase B (PKB); **AKT1:** AKT Serine/Threonine Kinase 1; **AKT2:** AKT Serine/Threonine Kinase 2; **AKT3:** AKT Serine/Threonine Kinase 3; **ATX:** Astaxanthin; **Bad:** B-cell Lymphoma-2 Gene-associated Promoter; **BATMAN-TCM:** Bioinformatics Analysis Tool for Molecular Mechanism of Traditional Chinese Medicine; **Bcl-2:** B-cell Leukemia/Lymphoma 2 Protein; **BP:** Biological Processes; **CC:** Cellular Components; **CTD:** Comparative Toxicogenomic Database; **CVD:** Cardiovascular Disease; **DAVID:** Database for Annotation, Visualization, and Integrated Discovery; **DNA:** Deoxyribonucleic Acid; **eNOS:** Endothelial Nitric Oxide Synthase**; ERK1:** Extracellular Signal-regulated Kinase 1; **F10:** Thrombin and Coagulation Factor X; **FDR:** False Discovery Rate; **FoxO:** Forkhead Box Transcription Factors; **GAD:** Genetic Association Database; **GO:** Gene Ontology; **GSK-3:** Glycogen Synthase Kinase-3; **HDAC1:** Histone Deacetylase 1; **HIF-1:** Hypoxia-inducible Factor-1; **HSP90AB1:** Heat Shock Protein 90 Alpha Family Class B Member 1; **HUVEC:** Human Umbilical Vein Endothelial Cells; **IKBα:** Inhibitor of Nuclear Factor Kappa-B Kinase Subunit Alpha; **IL:** Interleukins; **InChI:** International Chemical Identifier; **KEGG:** Kyoto Encyclopedia of Genes and Genomes; **KLF5:** Krueppel-like Factor 5; **LDL:** Low-Density Lipoprotein; **LPS:** Lipopolysaccharide; **M1:** M1 Macrophages; **MAPK3:** Mitogen-activated Protein Kinase 3; **MCODE:** Molecular Complex Detection; **MF:** Molecular Function; **miR-410:** MicroRNA 410; **MTOR:** Mammalian Target of Rapamycin; **NET:** Neutrophil Extracellular Trap; **NF-κB:** Nuclear Factor-kappa B**; NR:** Nuclear Receptor; **OMIM:** Online Mendelian Inheritance in Man; **ox-LDL:** Oxidized Low-Density Lipoprotein; **PDB ID:** Protein Data Bank ID; **PI3K-Akt:** Phosphoinositide 3-kinase-Protein Kinase B; **PIK3R1:** Phosphoinositide-3-kinase Regulatory Subunit 1; **PIK3R1/Akt:** Phosphoinositide-3-kinase (PI3K) Regulatory Subunit 1/Protein Kinase B; **PPI:** Protein–Protein Interaction; **PROC:** Protein C, Inactivator of Coagulation Factors Va And viiia; **RAGE:** Receptor for Advanced Glycation Endproducts; **RNA:** Ribonucleic Acid; **RXR:** Retinoid X Receptor; **RXRA:** Retinoid X Receptor Alpha; **RXRB:** Retinoid X Receptor Beta; **SMILES:** Simplified Molecular Input Line Entry Specification; **SRC:** Steroid Receptor Coactivator; **STRING:** Search Tool for the Retrieval of Interacting Genes; **TGF:** Transforming Growth Factor; **TNF:** Tumor Necrosis Factor; **WHO:** World Health Organization.

FUNDING

This study was supported by the University Grant, Geran Putra – Inisiatif Putra Siswazah (GP-IPS/2017/9576700) under Universiti Putra, Malaysia.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ACKNOWLEDGEMENT

The authors would like to acknowledge Universiti Putra Malaysia for awarding the grant (GP-IPS/2017/9576700).

AUTHOR CONTRIBUTIONS

Conceptualization, A.Z.M.B., A.N.S.S.A., M.F.S.S., M.N.H.A., V.L. and Y.K.Y.; Methodology, A.Z.M.B., A.N.S.S.A., M.F.S.S., M.N.H.A., V.L. and Y.K.Y.; Validation, V.L. and Y.K.Y.; Formal Analysis, A.Z.M.B., A.N.S.S.A., M.F.S.S., M.N.H.A., V.L. and Y.K.Y.; Investigation, A.Z.M.B., A.N.S.S.A., M.F.S.S., M.N.H.A., V.L. and Y.K.Y; Data Curation, A.Z.M.B., A.N.S.S.A., M.F.S.S.; Writing—Original Draft Preparation, A.Z.M.B., and Y.K.Y.; Writing—Review and Editing, A.Z.M.B., M.N.H.A., V.L. and Y.K.Y.; Supervision, Y.K.Y.; Project Administration, A.Z.M.B., and Y.K.Y.; Funding Acquisition, Y.K.Y. All authors have read and agreed to the published version of the manuscript.

REFERENCES

1. Palasubramaniam, J., Wang, X., & Peter, K. Myocardial Infarction-From Atherosclerosis to Thrombosis: Uncovering new diagnostic and therapeutic approaches. Arteriosclerosis, Thrombosis, and Vascular Biology, 2019;39(8):E176-85. https://doi.org/10.1161/ATVBAHA.119.312578
2. Bentzon, J. F., Otsuka, F., Virmani, R., & Falk, E. Mechanisms of Plaque Formation and Rupture. Circulation Research, 2014;114(12):1852-66. https://doi.org/10.1161/CIRCRESAHA.114.302721
3. Elvira-Torales, L. I., García-Alonso, J., & Periago-Castón, M. J. Nutritional Importance of Carotenoids and Their Effect on Liver Health: A Review. Antioxidants, 2019;8(7):229. https://doi.org/10.3390/ANTIOX8070229
4. Meléndez-Martínez, A. J., Mandić, A. I., Bantis, F., Böhm, V., Borge, G. I. A., Brnčić, M., *et al.* A comprehensive review on carotenoids in foods and feeds: status quo, applications, patents, and research needs. Critical Reviews in Food Science and Nutrition, 2022;62(8):1999-2049. https://doi.org/10.1080/10408398.2020.1867959
5. Guerin, M., Huntley, M. E., & Olaizola, M. Haematococcus astaxanthin: applications for human health and nutrition. Trends in Biotechnology, 2003; 21(5):P210-6. https://doi.org/10.1016/S0167-7799(03)00078-7
6. Snell, T. W., & Carberry, J. Astaxanthin bioactivity is determined by stereoisomer composition and extraction method. Nutrients, 2022;14(7):1522. https://doi.org/10.3390/NU14071522
7. Fakhri, S., Abbaszadeh, F., Dargahi, L., & Jorjani, M. Astaxanthin: A mechanistic review on its biological activities and health benefits. Pharmacological Research, 2018;136:1-20. https://doi.org/10.1016/J.PHRS.2018.08.012
8. Kohandel, Z., Farkhondeh, T., Aschner, M., Pourbagher-Shahri, A. M., & Samarghandian, S. Anti-inflammatory action of astaxanthin and its use in the treatment of various diseases. Biomedicine & Pharmacotherapy, 2022;145:112179. https://doi.org/10.1016/J.BIOPHA.2021.112179
9. Pereira, C. P. M., Souza, A. C. R., Vasconcelos, A. R., Prado, P. S., & Name, J. J. Antioxidant and anti-inflammatory mechanisms of action of astaxanthin in cardiovascular diseases (Review). International Journal of Molecular Medicine, 2021;47(1):37-48. https://doi.org/10.3892/IJMM.2020.4783
10. Wang, X., Wang, Z. Y., Zheng, J. H., & LI, S. TCM network pharmacology: A new trend towards combining computational, experimental and clinical approaches. Chinese Journal of Natural Medicines, 2021;19(1):1-11. https://doi.org/10.1016/S1875-5364(21)60001-8
11. Fan, J., Fu, A., & Zhang, L. Progress in molecular docking. Quantitative Biology, 2019;7(2):83-9. https://doi.org/10.1007/S40484-019-0172-Y/METRICS
12. Pinzi, L., & Rastelli, G. Molecular Docking: Shifting Paradigms in Drug Discovery. International Journal of Molecular Sciences, 2019;20(18):4331. https://doi.org/10.3390/IJMS20184331
13. Ferreira, L. G., Dos Santos, R. N., Oliva, G., & Andricopulo, A. D. Molecular Docking and Structure-Based Drug Design Strategies. Molecules, 2015;20(7):13384-421. https://doi.org/10.3390/MOLECULES200713384
14. Szklarczyk, D., Kirsch, R., Koutrouli, M., Nastou, K., Mehryary, F., Hachilif, R., *et al.* The STRING database in 2023: protein-protein association networks and functional enrichment analyses for any sequenced genome of interest. Nucleic Acids Research, 2023;51(D1):D638-46. https://doi.org/10.1093/NAR/GKAC1000
15. Sherman, B. T., Hao, M., Qiu, J., Jiao, X., Baseler, M. W., Lane, H. C., *et al.* DAVID: a web server for functional enrichment analysis and functional annotation of gene lists (2021 update). Nucleic Acids Research, 2022;50(W1):W216-21. https://doi.org/10.1093/NAR/GKAC194
16. Mir, W. R., Bhat, B. A., Kumar, A., Dhiman, R., Alkhanani, M., Almilaibary, A., *et al.* Network pharmacology combined with molecular docking and *in vitro* verification reveals the therapeutic potential of Delphinium roylei munz constituents on breast carcinoma. Frontiers in Pharmacology, 2023;14:1135898. https://doi.org/10.3389/FPHAR.2023.1135898
17. Dose, J., Matsugo, S., Yokokawa, H., Koshida, Y., Okazaki, S., Seidel, U., *et al.* Free Radical Scavenging and Cellular Antioxidant Properties of Astaxanthin. International Journal of Molecular Sciences, 2016;17(1):103. https://doi.org/10.3390/IJMS17010103
18. Farruggia, C., Kim, M. B., Bae, M., Lee, Y., Pham, T. X., Yang, Y., *et al.* Astaxanthin exerts anti-inflammatory and antioxidant effects in macrophages in NRF2-dependent and independent manners. The Journal of Nutritional Biochemistry, 2018;62:202-9. https://doi.org/10.1016/J.JNUTBIO.2018.09.005
19. Zuluaga, M., Gueguen, V., Letourneur, D., & Pavon-Djavid, G. Astaxanthin-antioxidant impact on excessive Reactive Oxygen Species generation induced by ischemia and reperfusion injury. Chemico-Biological Interactions, 2018;279:145-58. https://doi.org/10.1016/J.CBI.2017.11.012
20. Iwamoto, T., Hosoda, K., Hirano, R., Kurata, H., Matsumoto, A., Miki, W., *et al.* Inhibition of low-density lipoprotein oxidation by astaxanthin. Journal of Atherosclerosis and Thrombosis, 2000;7(4):216-22. https://doi.org/10.5551/JAT1994.7.216
21. Zou, T. Bin, Zhu, S. S., Luo, F., Li, W. Q., Sun, X. R., *et al.* Effects of Astaxanthin on Reverse Cholesterol Transport and Atherosclerosis in Mice. BioMed Research International, 2017; 2017:4625932. https://doi.org/10.1155/2017/4625932
22. Medina, I., Cougoule, C., Drechsler, M., Bermudez, B., Koenen, R. R., Sluimer, J., *et al.* Hck/Fgr kinase deficiency reduces plaque growth and stability by blunting monocyte recruitment and intraplaque motility. Circulation, 2015;132(6):490-501. https://doi.org/10.1161/CIRCULATIONAHA.114.012316
23. Speranza, L., Pesce, M., Patruno, A., Franceschelli, S., De Lutiis, M. A., Grilli, A., *et al.* Astaxanthin Treatment Reduced Oxidative Induced Pro-Inflammatory Cytokines Secretion in U937: SHP-1 as a Novel Biological Target. Marine Drugs, 2012;10(4):890-9. https://doi.org/10.3390/MD10040890
24. Jiang, B.H., & Liu, L.Z. AKT signaling in regulating angiogenesis. Current Cancer Drug Targets, 2008;8(1):19-26. https://doi.org/10.2174/156800908783497122
25. Zhai, C., Cheng, J., Mujahid, H., Wang, H., Kong, J., Yin, Y., *et al.* Selective Inhibition of PI3K/Akt/mTOR Signaling Pathway Regulates Autophagy of Macrophage and Vulnerability of Atherosclerotic Plaque. PLoS ONE, 2014;9(3):e90563. https://doi.org/10.1371/JOURNAL.PONE.0090563
26. Zarneshan, S. N., Fakhri, S., Farzaei, M. H., Khan, H., & Saso, L. Astaxanthin targets PI3K/Akt signaling pathway toward potential therapeutic applications. Food and Chemical Toxicology, 2020;145:111714. https://doi.org/10.1016/J.FCT.2020.111714
27. Li, H., Song, D., Liu, Q., Li, L., Sun, X., Guo, J., *et al.* miR-351 promotes atherosclerosis in diabetes by inhibiting the ITGB3/PIK3R1/Akt pathway and induces endothelial cell injury and lipid accumulation. Molecular Medicine, 2022;28:120. https://doi.org/10.1186/S10020-022-00547-9/FIGURES/11
28. Lou, X., Ma, X., Wang, D., Li, X., Sun, B., Zhang, T., *et al.* Systematic analysis of long non-coding RNA and mRNA expression changes in ApoE-deficient mice during atherosclerosis. Molecular and Cellular Biochemistry, 2019;462:61-73. https://doi.org/10.1007/S11010-019-03610-Y/FIGURES/8
29. Curek, G. D., Cort, A., Yucel, G., Demir, N., Ozturk, S., Elpek, G. O., *et al.* Effect of astaxanthin on hepatocellular injury following ischemia/reperfusion. Toxicology, 2010;267(1-3):147-53. https://doi.org/10.1016/J.TOX.2009.11.003
30. Li, J., Wang, F., Xia, Y., Dai, W., Chen, K., Li, S., *et al.* Astaxanthin Pretreatment Attenuates Hepatic Ischemia Reperfusion-Induced Apoptosis and Autophagy via the ROS/MAPK Pathway in Mice. Marine Drugs. 2015;13(6):3368-87. https://doi.org/10.3390/MD13063368
31. Li, M. Y., Sun, L., Niu, X. T., Chen, X. M., Tian, J. X., Kong, Y. Di, *et al.* Astaxanthin protects lipopolysaccharide-induced inflammatory response in Channa argus through inhibiting NF-κB and MAPKs signaling pathways. Fish and Shellfish Immunology, 2019;86:280-6. https://doi.org/10.1016/j.fsi.2018.11.011
32. Lim, S. R., Kim, D. W., Sung, J., Kim, T. H., Choi, C. H., & Lee, S. J. Astaxanthin inhibits autophagic cell death induced by bisphenol a in human dermal fibroblasts. Antioxidants, 2021;10(8):1273. https://doi.org/10.3390/antiox10081273
33. Nan, S., Wang, Y., Xu, C., & Wang, H. Interfering microRNA-410 attenuates atherosclerosis via the HDAC1/KLF5/IKBα/NF-κB axis. Molecular Therapy-Nucleic Acids, 2021;24:P646-57. https://doi.org/10.1016/j.omtn.2021.03.009

34. Venkidasamy, B., Thiruvengadam, M., Thirupathi, P., & Subramanian, U. Inhibition of histone deacetylases is the major pathway mediated by astaxanthin to antagonize LPS-induced inflammatory responses in mammary epithelial cells. Journal of Biochemical and Molecular Toxicology, 2020;34(8):e22507. https://doi.org/10.1002/JBT.22507
35. Dawson, M. I., & Xia, Z. The Retinoid X Receptors and Their Ligands. Biochimica et Biophysica Acta, 2012;1821(1):21-56. https://doi.org/10.1016/j.bbalip.2011.09.014
36. Roszer, T., Menéndez-Gutiérrez, M. P., Cedenilla, M., & Ricote, M. Retinoid X receptors in macrophage biology. Trends in Endocrinology and Metabolism, 2013; 24(9):P460-8. https://doi.org/10.1016/j.tem.2013.04.004
37. Gaggini, M., Gorini, F., & Vassalle, C. Lipids in Atherosclerosis: Pathophysiology and the Role of Calculated Lipid Indices in Assessing Cardiovascular Risk in Patients with Hyperlipidemia. International Journal of Molecular Sciences, 2022;24(1):75. https://doi.org/10.3390/IJMS24010075
38. Qi, H., Yang, S., & Zhang, L. Neutrophil Extracellular Traps and Endothelial Dysfunction in Atherosclerosis and Thrombosis. Frontiers in Immunology, 2017;8:928. https://doi.org/10.3389/FIMMU.2017.00928
39. Cunningham, K. S., & Gotlieb, A. I. The role of shear stress in the pathogenesis of atherosclerosis. Laboratory Investigation, 2005;85(1):9-23. https://doi.org/10.1038/labinvest.3700215
40. Ichiki, T. Thyroid hormone and atherosclerosis. Vascular Pharmacology, 2010;52(3-4):151-6. https://doi.org/10.1016/J.VPH.2009.09.004.

Cite this article: Barizi AZM, Azman ANSS, Saad MFS, Abdullah MNH, Lim V, Yong YK. Network Pharmacology and Molecular Docking Approaches of Astaxanthin (ATX) against Atherosclerosis. Pharmacog Res. 2024;16(3):626-37.