To explore the mechanism of Naodesheng tablets in the treatment of atherosclerosis based on network pharmacology and bioinformatics

Peng-Yu Wang1, Jia-Hui Hu1, Shuo Zhang1, Zhuo-Ling Chu1, You-Zhi Zhang1 *

1 School of Pharmacy, Xianning Medical College, Hubei University of Science and Technology, Xianning 437100, China.

*Corresponding to: You-Zhi Zhang, School of Pharmacy, Xianning Medical College, Hubei University of Science and Technology, NO.88, Xianning Street, Xianan District, Xianning 437100, China. E-mail: yzzhang242@hbust.edu.cn.

Abstract

Background: Naodesheng tablets (NDST) was widely used in clinical treatment as a drug for cardiovascular diseases, but the mechanism for treating atherosclerosis was unknown. On the basis of the network pharmacology and bioinformatics, predict the relevant targets and signalling pathways for NDST in the treatment of atherosclerosis. Methods: First, the targets of NDST and the targets for treating atherosclerosis were looked for in different databases. Next, Venny 2.1.0 was used to find the genes that overlapped between NDST and targets for treating atherosclerosis. Subsequently, the herb-active ingredient-target-disease were obtained to explore the hub compound. Moreover, the Metascape database was used for Gene Ontology and Kyoto Encyclopedia of Genes and Genomes enrichment analysis. And we constructed the “active ingredient-intersection target-pathway” network by Cytoscape software to gain the hub genes and pathways. Finally, molecular docking was used to verify the affinity of hub ingredients and hub genes. Results: In the results, 67 active ingredients and 322 targets of NDST were selected in ingredients-targets network. 154 overlapping targets of NDST (322) and atherosclerosis (1330) were obtained. Then, the herb-active ingredient-target-disease showed that quercetin, apigenin and luteolin were the hub ingredients to treat atherosclerosis. Further, the hub genes (PTGS2, RXRA, CASP3) and pathways (lipid and atherosclerosis) were accessed in active ingredient-intersection target-pathway. Finally, the results indicated that quercetin, apigenin and luteolin were better binding the PTGS2, RXRA, CASP3, especially PTGS2 and luteolin in molecular docking. Conclusion: In conclusion, quercetin, apigenin and luteolin, as the main ingredients of NDST could play an important role in PTGS2, RXRA, and CASP3 for treating atherosclerosis via the lipid and atherosclerosis (TNF signaling pathway).

Keywords: Naodesheng tablets; atherosclerosis; network pharmacology; bioinformatics; molecular docking
**Introduction**

In recent years, the global mortality rate due to atherosclerotic diseases has been significant, with approximately 20 million people losing their lives [1]. Although advancements in modern medical treatments, such as medication, surgery, and interventions, have led to a decrease in mortality, the incidence rate of atherosclerotic diseases continues to rise. Atherosclerosis primarily affects middle-aged and elderly individuals over the age of 40, and its progression accelerates after the age of 49 [2]. Men have a higher incidence rate compared to premenopausal women, but after menopause, the incidence rates become similar between both sexes. Research suggests that estrogen, a hormone found in higher levels in premenopausal women, possesses anti-atherosclerotic properties, which might contribute to the gender differences in incidence [3]. A concerning trend is the increasing age of onset of atherosclerosis, with autopsies revealing early atherosclerotic lesions in some young adults and even children. This highlights the importance of preventive measures and lifestyle modifications from an early age to mitigate the risk of disease progression [4].

In summary, while the mortality rate of atherosclerotic diseases has been decreasing due to advancements in medical treatments, the incidence rate continues to rise. Atherosclerosis primarily affects middle-aged and elderly individuals, with notable gender differences that may be linked to the protective effects of estrogen. The emergence of atherosclerotic lesions in younger individuals emphasizes the need for early prevention and intervention strategies.

Naodesheng tablets (NDST) are made from herbs and are known for their positive effects on blood circulation, getting rid of blood clots, and opening pathways and collaterals [5]. The tablets consist of several key ingredients, including Radix et Rhizoma Notoginseng, Chuanxiong Rhizoma, Carthami Flos, Puerae Radix and Crateae Fructus. These ingredients work synergistically to address various health conditions. The primary application of NDST is for dizziness and stroke caused by blood stasis obstructing collateral. This condition is often characterized by limb paralysis, speech difficulties, vertigo, and blurred vision. Additionally, the tablets are used for managing cerebral atherosclerosis, ischemic stroke, and the sequelae of cerebral hemorrhage [6]. Notoginseng Radix et Rhizoma, one of the essential ingredients in NDST, serves a vital role in promoting blood circulation and eliminating blood clots. It is combined with Carthami Flos to further enhance blood circulation, remove blood stasis, and activate collateral [7]. By employing the synergistic effects of the individual components, these tablets effectively improve blood flow, relieve pain, and enhance overall well-being.

Network pharmacology, a burgeoning discipline rooted in systems biology, offers a powerful approach to analyzing biological systems networks. By examining these networks, specific signaling nodes can be identified for the design of multi-target drugs [8]. This field places significant emphasis on the multi-pathway regulation of signaling pathways, aiming to enhance drug therapeutic efficacy and minimize toxic side effects. Consequently, it can make clinical research on new drugs more likely to succeed and reduce the cost of manufacturing new drugs [9]. The purpose of this study is to learn more about the important targets and signaling pathways connected to NDST in light of the benefits provided by network pharmacology. It serves as a resource for further investigation into the therapeutic impact and mechanism of action of this drug.

In this study, the comprehensive approach was employed to investigate the potential effects of NDST for atherosclerosis. As shown in Figure 1, the research combined network pharmacology methods and bioinformatics technology to identify and analyze the effective ingredients, targets, and pathways associated with NDST. Furthermore, in order to investigate the biological processes, functions, and pathways linked to the indicated targets, the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were carried out. GO enrichment analysis categorized gene functions, while KEGG enrichment analysis provided insights into the involvement of genes in different biological pathways. To validate and visualize the interactions between the hub compounds and hub genes, molecular docking technology was employed.

**Materials and methods**

**Software and databases**

The study was constructed based on the softwares, website and databases as follows: AutoDock [1.5.7]; Cytoscape 3.9.0; Pynol 2.5; Traditional Chinese Medicine Database and Analysis Platform (TCMSP, https://tcmsp-e.com); GeneCards (https://www.genecards.org); Gene Expression Omnibus database (GEO, https://www.ncbi.nlm.nih.gov/geo/); Online Mendelian Inheritance in Man (OMIM, https://www.omim.org); Therapeutic Target Database (http://dd.idrblab.net/ttd); Uniprot (http://www.uniprot.org); Venny 2.1.0 (http://bioinfogp.cnb.csic.es); STRING (http://string-db.org); SRplot (www.bioinformatics.com.cn); Metascape (https://metascape.org); Protein Data Bank (https://www.rcsb.org/); Pubchem (https://pubchem.ncbi.nlm.nih.gov/); KEGG (https://www.genome.jp/).

![Figure 1 The overall design flowchart of this study. NDST, Naodesheng tablets; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.](https://www.trmjournals.com/pmr)
Construction of ingredients-targets network of NDST
The TCMSP, a comprehensive pharmacology database and analysis platform for traditional Chinese medicine, was used to determine the components and matching targets of NDST. The main ingredients of the tablets were *Notoginseng Radix et Rhizoma*, *Chuanxiong Rhizoma*, *Carthami Flos*, *Puerariae Radix* and *Crataegi Fructus*. The keywords were searched for main ingredients and then the information with oral bioavailability ≥ 20% and drug-likeness ≥ 0.1 was screened. Meanwhile, the Uniprot database (protein sequence and functional information resource database) was applied to match the active ingredient targets with gene names and remove duplicate values to obtain the targets of NDST. Finally, the data were uploaded to cytoscape 3.9.0 software to draw the active ingredient-target network.

Screening the targets of atherosclerosis and constructing the herb-active ingredient-target-diseases network
The disease databases of GeneCards, Online Mendelian Inheritance in Man and Therapeutic Target Database were searched with “atherosclerosis” as the keyword, and the median relevance score of the data obtained from GeneCards was calculated twice. The median relevance scores of GeneCards were 0.67 and 1.31, respectively, and the disease targets with median scores greater than or equal to 1.31 were selected. Meanwhile, the GEO2R were used to explore the data in GSE111782. The [log2FC] ≥ 1 was set to select the differential genes. Finally, the targets were obtained by taking the concatenation of the differential genes (30 up-regulated genes and 30 down-regulated genes) with the disease targets filtered from the three databases and removing the duplicates. The targets of NDST and atherosclerosis were imported into Venny 2.1.0, and the overlapped genes of them was obtained. Subsequently, the relevant data were uploaded into cytoscape 3.9.0 software to create the herb-active ingredient-target-diseases network.

Active ingredient-intersection target-pathway network was performed to obtain the hub targets and pathways
To further explore the function of intersecting genes for NDST and atherosclerosis, KEGG enrichment pathways and GO biofunctional analysis were performed using Metascape database. First, the overlapped genes were imported to Metascape, and the species was limited to “Homo Sapiens”. Then, the top ten pathways of KEGG enrichment were obtained and we also obtained five common groups of entries: biological process (BP), cellular component (CC), and molecular function (MF) by “count value”. Finally, the data was visualized in the online mapping tool of “SRplot”. Meanwhile, the relevant data was import into cytoscape 3.9.0 software to make the ingredient-targets-pathway network, especially applying the “Analyze Network” tool for visualization.

Molecular docking of hub ingredients and hub targets
The top three targets (PTG52, RXRA, and CASP3) with degree were screened in Cytoscape 3.9.0 software that were used as docking receptors, and the top three active ingredients quercetin, apigenin, and luteolin were the ligand, which were docked by AutoDock 1.5.7.

Results
Screening of active compounds and targets of NDST
First, the TCMSP database was used to discover the active components and the related targets of NDST, *Notoginseng Radix et Rhizoma*, *Chuanxiong Rhizoma*, *Carthami Flos*, *Puerariae Radix* and *Crataegi Fructus* as the keywords. Then, the information with oral bioavailability ≥ 20% and drug-likeness ≥ 0.1 were screened out, and 19, 21, 31, 7 and 7 active ingredients were screened out respectively. The TCMSP database was used to obtain 67 active ingredients (the herb names and compounds have been renamed in the annexed table) and their corresponding 1680 targets of NDST. The targets of the ingredients were matched with the human gene names using the Uniprot database, and the duplicate values were removed, and 322 targets were successfully matched.

Construction of active ingredient-targets network of NDST
The data were uploaded to cytoscape 3.9.0 software and the “Analyze Network” tool in cytoscape 3.9.0 software was used to visualize the data (Figure 2), which shows that the five circles represent the five primary categories of active ingredients in NDST, with a total of 67 active ingredients. The bottom row represents the common ingredients. The blue section in the middle represents the 322 corresponding targets of the active constituents in NDST, and the pattern’s color tones are ordered by their degree value. According to Figure 2 Active ingredient-target network of Naodesheng tablets. The five circles around the figure were the five categories of active ingredients of Naodesheng tablets, the bottom row is the common ingredients (A–K), the total number of active ingredients was 67, the middle blue part represented the corresponding targets (322) of the active ingredients of Naodesheng tablets, the pattern color shades were arranged in degree value.
the degree value, the top ten components network nodes can be obtained (Table 1) and the top three active ingredients of NDST with the most corresponding targets are MOL000098 (quercetin), MOL000008 (apigenin) and MOL000422 (kaempferol), with degree values of 153, 81 and 63, respectively, indicating that these three ingredients have a high correlation with the targets.

Table 1 The top 10 ingredients of Naodesheng tablets

<table>
<thead>
<tr>
<th>Name</th>
<th>MOL ID</th>
<th>Compound</th>
<th>OB</th>
<th>DL</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>MOL000098</td>
<td>quercetin</td>
<td>46.43</td>
<td>0.28</td>
<td><em>Puerariae Radix, Notoginseng Radix et Rhizoma, Carthami Flos</em></td>
</tr>
<tr>
<td>HH22</td>
<td>MOL000008</td>
<td>apigenin</td>
<td>23.06</td>
<td>0.21</td>
<td><em>Carthami Flos</em></td>
</tr>
<tr>
<td>J</td>
<td>MOL000422</td>
<td>kaempferol</td>
<td>41.88</td>
<td>0.24</td>
<td><em>Puerariae Radix, Notoginseng Radix et Rhizoma, Carthami Flos</em></td>
</tr>
<tr>
<td>HH16</td>
<td>MOL000006</td>
<td>luteolin</td>
<td>36.16</td>
<td>0.25</td>
<td><em>Carthami Flos</em></td>
</tr>
<tr>
<td>GG1</td>
<td>MOL012297</td>
<td>puerarin</td>
<td>24.03</td>
<td>0.69</td>
<td><em>Puerariae Radix</em></td>
</tr>
<tr>
<td>C</td>
<td>MOL000675</td>
<td>oleic acid</td>
<td>33.13</td>
<td>0.14</td>
<td><em>Puerariae Radix, Notoginseng Radix et Rhizoma, Carthami Flos</em></td>
</tr>
<tr>
<td>B</td>
<td>MOL000358</td>
<td>beta-sitosterol</td>
<td>36.91</td>
<td>0.75</td>
<td><em>Puerariae Radix, Notoginseng Radix et Rhizoma, Carthami Flos</em></td>
</tr>
<tr>
<td>GG5</td>
<td>MOL000392</td>
<td>formononetin</td>
<td>69.67</td>
<td>0.21</td>
<td><em>Puerariae Radix</em></td>
</tr>
<tr>
<td>HH18</td>
<td>MOL002714</td>
<td>baicalin</td>
<td>33.52</td>
<td>0.21</td>
<td><em>Carthami Flos</em></td>
</tr>
<tr>
<td>SZ2</td>
<td>MOL000354</td>
<td>isorhamnetin</td>
<td>49.60</td>
<td>0.31</td>
<td><em>Crataegi Fructus</em></td>
</tr>
</tbody>
</table>

Red squares indicate oxygen bonding and green squares indicate hydrogen bonding. OB, oral bioavailability; DL, drug-likeness.
Construct the overlapping targets of NDST and atherosclerosis

GeneCards, OMIM, and DrugBank databases were utilised to derive 1221, 144, and 46 targets, respectively. Currently, the GSE11782 from the GEO database was selected, and differential genes were depicted using a volcano plot (Figure 3A). Moreover, 60 significantly different genes (30 up-regulated and 30 down-regulated) were obtained by using GEO2R online analysis, which were combined and de-weighted to get the atherosclerosis targets (1330). The overlapping targets (154) of NDST and atherosclerosis were taken. Using Veeny 2.1.0, the green represented the 322 active ingredient targets of NDST, the green color represented the 1330 disease targets, the middle of green and blue included the 154 overlapping targets of NDST and atherosclerosis, as shown in Figure 3B.

Building the “herb-active ingredient-target-disease” network

Further, in order to acquire the hub components of NDST to treat atherosclerosis. The relevant data were uploaded to cytoscape 3.9.0 software to plot the herb-active ingredient-target-disease network. The data were visualized using the “Analyze Network” tool in cytoscape 3.9.0 software (Figure 4), and the pattern were arranged in order of degree value. The top three active ingredients are quercetin, apigenin and luteolin, and the top three targets were PTGS2, PTGS1 and RXRA.

GO and KEGG enrichment analysis in overlapping targets

The 154 overlapping targets of active ingredients of NDST and atherosclerosis were uploaded to Metascape website, and the species was limited to “Homo Sapiens”, and the top ten entries of the obtained data by count value were used to make KEGG enrichment graphs. In the KEGG analysis, the top three pathways with the highest enrichment significance were lipid and atherosclerosis, fluid shear stress and atherosclerosis, and AGE-RAGE signaling pathway in diabetic complications (Figure 5A).

We also obtained three groups of GO enrichment: BP, CC, and MF. Then, three groups of data by taking the top 5 pathways were visualized by count value. GO enrichment showed that BP was mainly focused on the response to hormone, response to peptide and positive regulation of protein phosphorylation; CC mainly included on membrane raft, membrane microdomain and side of membrane; MF mainly concentrated on kinase binding, DNA-binding transcription factor binding and transcription factor binding (Figure 5B).

Figure 3 The overlapping targets of NDST and atherosclerosis was constructed. (A) In the volcano plot, the horizontal coordinates indicate the value of log2FC. The significance size of the differential genes, log2FC the larger the value, the more significance were shown. Each points in the diagram indicates a gene, red is the 40 up-regulated genes significantly and blue is a significantly 40 down-regulated genes. (B) Green circles represented 322 genes of NDST, blue circles represented 1330 atherosclerosis genes and the intermediate overlap of 154 overlapping genes. NDST, Nao de Sheng tablets; AS, atherosclerosis.

Figure 4 Construction of “herb-active ingredient-target-disease” network. The orange triangle at the top of the graph is the atherosclerosis, the diamond in the middle in a rectangular arrangement is the gene target, and a total of five circular arrangements on the left and right as well as a horizontal row of parts below are the components of the drug. The size of the graphs was arranged in order of degree value.
Construction of active ingredient-intersection target-pathway network of NDST

The network diagram of the active ingredient-intersection target-pathway network was created using the “Analyze Network” tool (Figure 6). Based on the degree values, the top three active constituents were quercetin (63), apigenin (31), and luteolin (30) (Table 2). The top three targets were: PTGS2 (51), RXRA (22), RELA (18), and other targets degree values are shown in Table 3. In the Table 4, the top 3 pathways were lipid and atherosclerosis, fluid shear stress and atherosclerosis, and AGE-RAGE signaling pathway in diabetic complications. Furthermore, RXRA, CASP3, RELA and AKT1 were enriched in the lipid and atherosclerosis signaling pathway (Figure 7), which shows that the lipid and atherosclerosis signaling pathway is one of the important pathways to explore the mechanism of NDST treated atherosclerosis. In addition, TNF signaling pathway was in lipid and atherosclerosis as shown in Figure 7. Interestingly, PTGS2 was involved in TNF signaling pathway as shown in Figure 8.

Molecular docking

The hub ingredients were quercetin (H), apigenin (HH22), and luteolin (HH16), which were used in the treatment of atherosclerosis in NDST. They were docked with the hub targets (PTGS2, RXRA, and CASP3), as shown in Figure 9. According to the threshold values, nine sets of ligand-receptor docking results were obtained, and the binding energy was used to screen the targets with improved binding activity, where the ligands and targets had a binding energy of ~5 kcal/mol suggested that there was good binding activity [10]. The affinity of PTGS2 and apigenin (~6.36 kcal/mol) and luteolin (~6.30 kcal/mol) docking are relatively modest, according to Table 5. Especially, PTGS2 and apigenin were each connected by one hydrogen bond at SER-121, LYS-352, TYR-373, and GLN-370. PTGS2 and luteolin were linked by one hydrogen bond at LEU-366, LYS-369, and GLN-370, and by two hydrogen bonds at PHE-371. It demonstrated that PTGS2 was the predominant constituent.

Discussion

In this study, network pharmacology and bioinformatics methods were employed to obtain and analyze the targets of NDST, which are used in the treatment of atherosclerosis. First, utilizing a variety of pharmacological databases and the GEO database, 154 genes that overlapped with those of NDST and targets relevant to atherosclerosis were retrieved. Then, GO analysis and KEGG enrichment analysis showed that the lipid and atherosclerosis pathway was the most likely enriched pathway. Subsequently, the hub ingredients (quercetin, apigenin, and luteolin) and the hub genes (PTGS2, RXRA, and CASP3) were selected in ingredient-intersection target-pathway network. Finally, molecular docking verification showed that the hub ingredients had better binding abilities with the hub targets, especially PTGS2 and quercetin. This further illustrates that NDST may act on PTGS2, RXRA, and CASP3 to treat atherosclerosis.

Three hub ingredients (quercetin, apigenin, and luteolin) and three hub genes (PTGS2, RXRA, and CASP3) were screened using the network pharmacology and bioinformatics methodology. It was widely known that quercetin, apigenin, and luteolin as one of flavonoid, were found abundantly in fruits and vegetables [11]. And a lot of evidence indicated that quercetin, a natural polyphenol derived from human diet, effectively reduces inflammatory reactions and contributes to cardiovascular health [12, 13]. In addition, Luo et al. demonstrated that quercetin inhibits the p38 MAPK/p16 pathway to inhibit ox-LDL-induced senescence in plaque macrophages and attenuate atherosclerosis [14]. The flavonoid known as apigenin was later shown to be present in high quantities in fruits. It possesses a diverse spectrum of biological capabilities, some of which include the ability to combat inflammation, cancer, and bacteria [15]. Apigenin could also improve uncontrolled vasodilation and enhance the antioxidant activity of endothelial cells by up-regulating the activity of endothelial nitric oxide synthase and increasing the amount of nitric oxide and superoxide dismutase [16]. These effects have been reported to be beneficial in the treatment of atherosclerosis [17]. Meanwhile, luteolin was widely considered as a anti-inflammatory and exhibited anti obesity effects [18, 19]. The primary findings that were published in the papers that were collected demonstrated that luteolin has the ability to influence the pathways that are responsible for the initiation of inflammation. These pathways include toll-like receptors and high mobility group box-1 [20]. Therefore, quercetin, apigenin and luteolin have significant effects on the treatment of arteriosclerosis.

The hub genes (PTGS2, RXRA, and CASP3) were obtained in overlapping genes of NDST and atherosclerosis. In the results, they were predominantly membrane-distributed and perform roles in enzyme-factor binding and signal reception (Figure 5B). PTGS2, also COX2, was a cyclooxygenase expressed mainly in vascular luminal epithelial cells and is involved in prostaglandin synthesis [21]. It has been shown to be expressed mainly in inflammatory cell lines.
Figure 6 Active ingredient-intersection target-pathway network of Naodesheng tablets. The intensity of each hue and the magnitude of each node together represent the degree. The outer green circle in the figure is the ingredients of NDST, the middle purple triangle is the pathway, the middle yellow square was the pathways targets. NDST, Naodesheng tablets.

<table>
<thead>
<tr>
<th>Signal</th>
<th>Effective compound</th>
<th>Degree</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>quercetin</td>
<td>63</td>
</tr>
<tr>
<td>HH22</td>
<td>apigenin</td>
<td>31</td>
</tr>
<tr>
<td>HH16</td>
<td>luteolin</td>
<td>30</td>
</tr>
<tr>
<td>GG1</td>
<td>puerarin</td>
<td>29</td>
</tr>
<tr>
<td>J</td>
<td>kaempferol</td>
<td>21</td>
</tr>
<tr>
<td>HH18</td>
<td>baicalein</td>
<td>15</td>
</tr>
<tr>
<td>HH14</td>
<td>beta-carotene</td>
<td>11</td>
</tr>
<tr>
<td>SZ2</td>
<td>isorhamnetin</td>
<td>10</td>
</tr>
<tr>
<td>SQ3</td>
<td>oleic acid</td>
<td>8</td>
</tr>
<tr>
<td>GG5</td>
<td>formononetin</td>
<td>8</td>
</tr>
</tbody>
</table>

Table 2 The top 10 compounds in ingredient-intersection target-pathway network

<table>
<thead>
<tr>
<th>Gene</th>
<th>Degree</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTGS2</td>
<td>51</td>
</tr>
<tr>
<td>RXRA</td>
<td>22</td>
</tr>
<tr>
<td>CASP3</td>
<td>18</td>
</tr>
<tr>
<td>RELA</td>
<td>18</td>
</tr>
<tr>
<td>AKT1</td>
<td>16</td>
</tr>
<tr>
<td>BCL2</td>
<td>15</td>
</tr>
<tr>
<td>AR</td>
<td>14</td>
</tr>
<tr>
<td>PPARG</td>
<td>14</td>
</tr>
<tr>
<td>PIK3CG</td>
<td>13</td>
</tr>
<tr>
<td>TNF</td>
<td>13</td>
</tr>
</tbody>
</table>

Table 3 The top 10 genes in ingredient-intersection target-pathway network

<table>
<thead>
<tr>
<th>Pathway</th>
<th>Degree</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid and atherosclerosis</td>
<td>39</td>
</tr>
<tr>
<td>Fluid shear stress and atherosclerosis</td>
<td>35</td>
</tr>
<tr>
<td>AGE-RAGE signaling pathway in diabetic complications</td>
<td>33</td>
</tr>
<tr>
<td>PI3K-Akt signaling pathway</td>
<td>32</td>
</tr>
<tr>
<td>MAPK signaling pathway</td>
<td>29</td>
</tr>
<tr>
<td>Human cytomegalovirus infection</td>
<td>29</td>
</tr>
<tr>
<td>Kaposi sarcoma-associated herpexvirus infection</td>
<td>29</td>
</tr>
<tr>
<td>Hepatitis B</td>
<td>28</td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>26</td>
</tr>
<tr>
<td>Coronavirus disease - COVID-19</td>
<td>26</td>
</tr>
</tbody>
</table>
Figure 7 The KEGG lipid and atherosclerosis diagram. The color red signified the hub genes in lipid and atherosclerosis. RXRA, CASP3, RELA, AKT1 were involved in lipid and atherosclerosis. The TNF signing pathway in the lipid and atherosclerosis. KEGG, Kyoto Encyclopedia of Genes and Genomes.

Figure 8 The KEGG diagram of TNF signaling pathway. PTGS2 and CASP3 were involved in TNF signaling pathway. The red represented the hub genes in TNF signaling pathway. KEGG, Kyoto Encyclopedia of Genes and Genomes.
Figure 9 Molecular docking visualization of the hub ingredients and hub targets of Naodesheng tablets for the treatment of atherosclerosis. The cyan, pink and red molecular represents quercetin, apigenin and luteolin, and the gray, skyblue and green sections represent fragments of PTGS2, RXRA, and CASP3.

Table 5 The molecular docking of hub ingredients and hub genes (kcal/mol)

<table>
<thead>
<tr>
<th>Protein (PUB ID)</th>
<th>Protein Structure</th>
<th>Compound</th>
<th>Affinity (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTGS2 (5f19)</td>
<td></td>
<td>quercetin</td>
<td>-4.63</td>
</tr>
<tr>
<td></td>
<td></td>
<td>apigenin</td>
<td>-6.36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>luteolin</td>
<td>-6.30</td>
</tr>
<tr>
<td>RXRA (7pdq)</td>
<td></td>
<td>quercetin</td>
<td>-4.76</td>
</tr>
<tr>
<td></td>
<td></td>
<td>apigenin</td>
<td>-5.16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>luteolin</td>
<td>-4.77</td>
</tr>
<tr>
<td>CASP3 (5i9t)</td>
<td></td>
<td>apigenin</td>
<td>-5.53</td>
</tr>
<tr>
<td></td>
<td></td>
<td>luteolin</td>
<td>-5.59</td>
</tr>
</tbody>
</table>
atherosclerotic plaque formation [22], RXRA, also known as retinoid X receptor alpha, is a well-characterized mediator that controls lipid metabolism [23]. One of the early signs of atherosclerosis formation is the deposition of lipids on the vessel wall and the formation of foam cells [24]. It has been shown that RXRA plays an important role in cell proliferation, differentiation and death processes and is considered a major regulator of atherosclerosis, and after RXRA activation by ox-LDL, it may lead to atherosclerosis [25, 26]. According to studies, the activation of RXRA results in the accumulation of adipose in liver cells [27]. CASP3, an inflammatory mediator of apoptosis, was found to be significantly expressed during the pathological process of atherosclerosis [28, 29]. Qin et al. [30] indicated that apigenin has been shown to improve endothelial function through regulating oxygen species/caspase-3 and the nitric oxide pathway, as well as decrease the progression of atherosclerosis.

In addition, the findings of the KEGG enrichment project demonstrated that the pathway regulating lipids and atherosclerosis was the one that was significantly enriched the most, and the hub genes were enriched to this pathway, with PTGDS and CASP3 enriched to the TFN signaling pathway, a subpathway of the lipid and atherosclerosis pathway. It has been demonstrated that the TFN signaling pathway plays a significant role in the development of vascular endothelial hyperplasia [31]. When the upstream gene of PTGDS, TFN-a were inhibited, it could significantly reduce the occurrence of autophagy leading to inflammation [32], and it could effectively suppress neuroinflammation by inhibiting the TFN pathway [33]. Previous study reported that lignocaine could significantly reduce the expression of PTGDS induced by IL-1β for the purpose of treating inflammation [34]. Moreover, the development of atherosclerotic plaque in mice fed a high fructose diet was prevented by quercetin via PI3K/AKT and CASP3 activation, which was regulated by reactive oxygen species [35]. As a subpathway of lipid and atherosclerosis, TFN signaling pathway plays a significant role in the development of endothelial inflammation, which in turn is implicated in atherosclerosis.

In this study, a network pharmacology methodology was used to investigate the interactions between three primary active components (quercetin, apigenin, and luteolin), as well as three hub targets (PTGDS, RXRA and CASP3). It embodies the properties of network pharmacology, which include many ingredients, multiple targets, and multiple pathways, and it offers a theoretical foundation for additional research. However, there are still some limitations. The results of this paper are based on the literature, and some of the results still need to be confirmed by relevant experiments.

**Conclusion**

In conclusion, we hypothesized that quercetin, apigenin and luteolin, as the main ingredients of NDST, treat atherosclerosis via the lipid and atherosclerosis (TFN signaling pathway) of PTGDS, RXRA, and CASP3. The network pharmacology represents the features of multiple ingredients, multiple genes, and multiple pathways, and it may bring an innovative proposal for the treatment of atherosclerosis by using NDST.

**References**


