Original Article

Etiopathogenesis Of Microorganisms In Causing Atherosclerotic Plaques In Coronary Arteries And To Explore The Potential Targets Of Licorice In Treating Coronary Atherosclerosis

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Abstract

Relevance to ethnopharmacology: Licorice, one of the primogenital and greatest well-known herbal remedies, was been used for cardiovascular diseases, antiviral, antibacterial and has ethnopharmacological significance. Multiple investigations have demonstrated that the atherosclerotic plaque contains bacterial and viral microorganisms. Pathogens can replicate in cells like macrophages or remain dormant in order to cause a chronic inflammatory environment. According to recent research, supplementing hypercholesterolemic patients with licorice extracts prevented the onset of atherosclerosis (AS). Numerous studies have demonstrated that the primary active ingredients in licorice, licorice flavonoids, have a wide range of effects in term of pharmacology, including control over lipid metabolism, anti-inflammation, and antioxidation. Licorice's primary anti-AS components are still a mystery.

Aim of the study: This study aims look into the method underlying that underlie the active ingredients in licorice's anti-atherosclerotic effects.

Materials and methods: In order to determine the active components, potential targets, and molecular mechanisms of licorice associated with the treatment of AS, a network pharmacology approach including oral bioavailability (OB) prediction, protein-protein interaction (PPI) network construction and analysis, and Gene Ontology term and Genomes (KEGG) pathway analyses was used. The interactions between the active compounds and the underlying targets were examined using molecular docking analysis.

Results: Inclusive systems method successfully recognized 125 bioactive components from licorice as well as 39 potential targets for this medicinal herb. A number of diseases affecting the digestive organization, and respiratory organization are all closely linked to these 91 targets. To clarify the mechanism of action of this herbal remedy, in addition plots of these seeks are made for drug-target-disease networks.

Conclusion: In this article, the role of microorganisms in causing of atherosclerosis and its treatment by a novel medicine such as licorice by using in silico approach. This effort should aid in the understanding of specific apparatuses for herbal drugs and the sighting of new drugs derived from plants.

Introduction:

Coronary artery disease is a cardiovascular illness that results in coronary artery stenosis or occlusion and myocardial ischemia, hypoxia, or necrosis. CAD patients may experience luminal stenosis or occlusion due to inflammation, thrombogenesis, angiogenesis, apoptosis, atherosclerosis and other factors (Harsh Agrawal et

al.,2020; Hayato Tada et al.,2018). Globally, 17.3 million people passed away from cardiovascular diseases in 2008; 6.2 million of those deaths were due to stroke, and 7.3 million to coronary heart disease. According to statistics from the American Heart Association, CAD is the most common cause of death in countries that are both developed and developing, placing a heavy burden on people's health, finances, and emotional well-being on a global scale. In accordance to some research, a bad lifestyle, diabetes, hypertension and dyslipidemia are all hazard aspects for CAD (Doron Aronson et al.,2014, Thomas Weber et al.,2016)

Symptoms of CAD:

- Angina (chest pain or discomfort)
- Tiredness, dizziness, nausea (feeling sick to your stomach), or cold sweat
- Arm or shoulder pain or discomfort
- Breathing difficulties (www.cdc.gov/heartdisease/coronary_ad.htm, 03/06/23, 7:03pm)

The components that can be used to prevent, treat, and diagnose diseases are gathered, processed, and prepared using TCM theory, which is also used to describe the mechanism of action and direct application in medicine (David Cyranoski.,2018). CAD can be effectively treated with TCM as well. Li's research indicates that TCM may cure the CAD because of its anti-inflammatory properties. (Si-Ming Li et al.,2019).

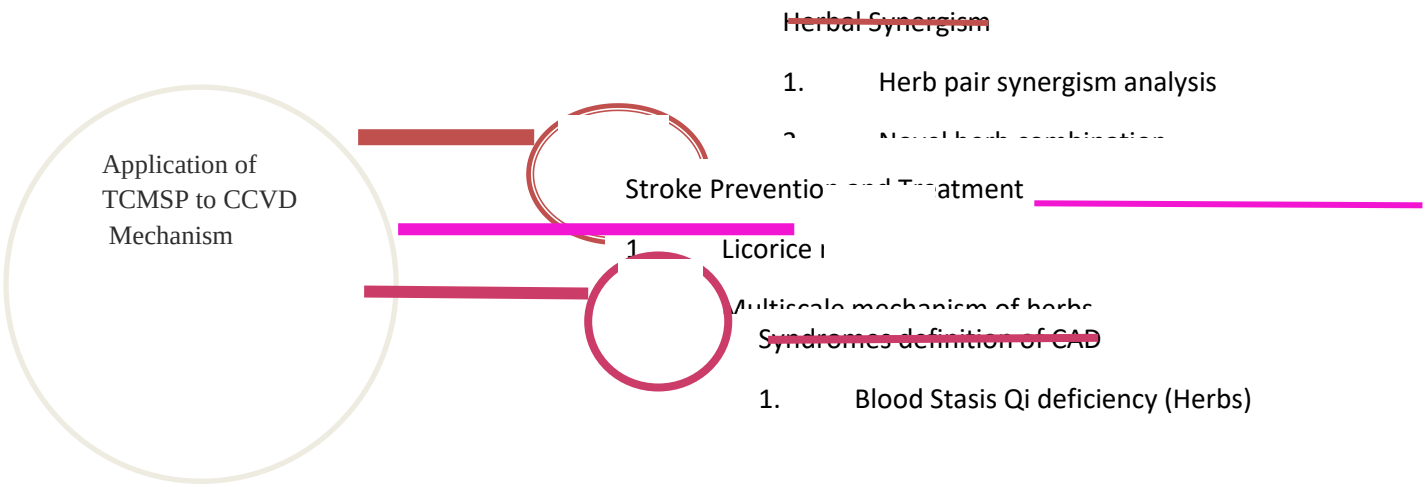

Fig 1: Application of TCMSP to CCVD mechanism and management.

A chronic inflammatory disease, atherosclerosis. Interest in a clinical association between these conditions was sparked by the pathophysiological similarities between chronic infections and atherosclerosis. Numerous bacteria and viruses have been shown to directly affect the vascular endothelium as well as indirectly through the release of systemic cytokines, both of which speed up the atherosclerotic process. Chlamydia pneumoniae, Herpes simplex virus, cytomegalovirus, hepatitis C virus, Measles, influenza A virus, HIV and Helicobacter pylori are just a few of the infectious agents that have been linked to atherosclerotic disease. However, multiple clinical trials demonstrating the ineffectiveness of anti-infective therapies in mitigating atherosclerotic cardiovascular events meant that this association did not meet the Koch's postulates of causation. The discovery of underlying pathophysiological mechanisms, as well as experience with vaccination against various infectious agents, has

opened up a new avenue of research for the development of an anti-atherosclerotic vaccine. Table 1 lists the major local pathogenic effects of various microbes (Naga Venkata K Pothineni et al.,2017).

Table:1. Summary of local pro atherosclerotic mechanisms in chronic infections

Phase of atherogenesis	Pathogen	Pathogenic effect
Lesion progression □ Proliferation of SMC	Cp, CMV Pg, CMV	1. Resistance to apoptosis 2. ↑ production of growth factors
Lesion instability 1. Rupture 2. Thrombosis	Cp, Hp Cp, HIV, CMV Cp, CMV, HSV	1. Altered vasomotor tone 2. ↑ production of MMPs 3. Anticoagulant → Pro-coagulant milieu

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Ancient herb licorice is well-known and frequently used in traditional Chinese medicine. Three original Glycyrrhiza inflata Bat, Glycyrrhiza uralensis Fisch and Leguminosae plants Glycyrrhiza glabra L, are recorded in the Chinese Pharmacopoeia as licorice (Hu He et al., 2019, Giulia Pastorino et al., 2018). Licorice is primarily used in indigenous remedy and medical training to cure Influenza, liver damage, cardiovascular diseases and HCV (Zihan Zheng et al., 2021, Zhixin Zhang et al., 2021, Lei Zhang et al., 2021). Licorice metabolites have a large area of pharmacological activities, including antitumor, anti-inflammatory, antiviral, antiatherosclerosis, antimicrobial and other activities, according to recent studies. An overview of licorice's antiviral and antimicrobial properties is given in this paper.

One of the important substances extracted from licorice roots is called GL. The antiviral activity of GL has been confirmed by numerous studies in recent years. According to Matsumoto et al.16, GL specifically targeted the release phase of the infection of cells by infectious anti-hepatitis C virus (HCV) particles. These results suggested that GL may play novel therapeutic roles in the care of people with chronic hepatitis C (Liqiang Wang et al.,2015). Licorice extracts supplementation for a month has been shown to have anti-atherosclerotic effects by raising plasma low-density lipoprotein cholesterol oxidation resistance and decreasing levels of plasma lipids (Bianca

Fuhrman et al., 2002). After a year of use, licorice extracts were found to slow the progression of atherosclerosis in patients with high cholesterol (Yacov Fogelman et al., 2016).

This study's objectives were to investigate the primary chemical constituents of licorice flavonoids as well as the mechanism of action in Atherosclerosis. The main active substances of licorice were initially screened for their potential signaling pathways in AS using network pharmacology.

Methodology:

Collection and screening of licorice compounds:

Licorice compound were gathered using the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (https://tcmspw.com/tcmsp.php). The candidate compounds had to meet the following parameters in order to be considered for further analysis: molecular weight (MW) 500, (DL) 0.1 and OB 20%. After screening, the targets were chosen based on bioactive ingredients (Zhi-Kun Qiu et al.,2021).

Target interactions between substances and disease:

To predict the possibility of licorice's pharmacological effects on Atherosclerosis, targets associated with licorice and Coronary Atherosclerosis should be obtained. Network pharmacology was used to perform the intersection (Zhi-Kun Qiu et al.,2021).

Network Analysis of PPI:

To find out more about how overlapping targets interact, the Stitch database (https://bio.tools/stitch) created a network of functional proteins. The desired minimum interchange score was set to 0.400 condensation medium, with the remaining confines left at their default values. After that, the PPI data was downloaded and displayed using Shiny GO v0.741 (http://bioinformatics.sdstate.edu/go74/) (Hafiza Ayesha Andleeb et al., 2022).

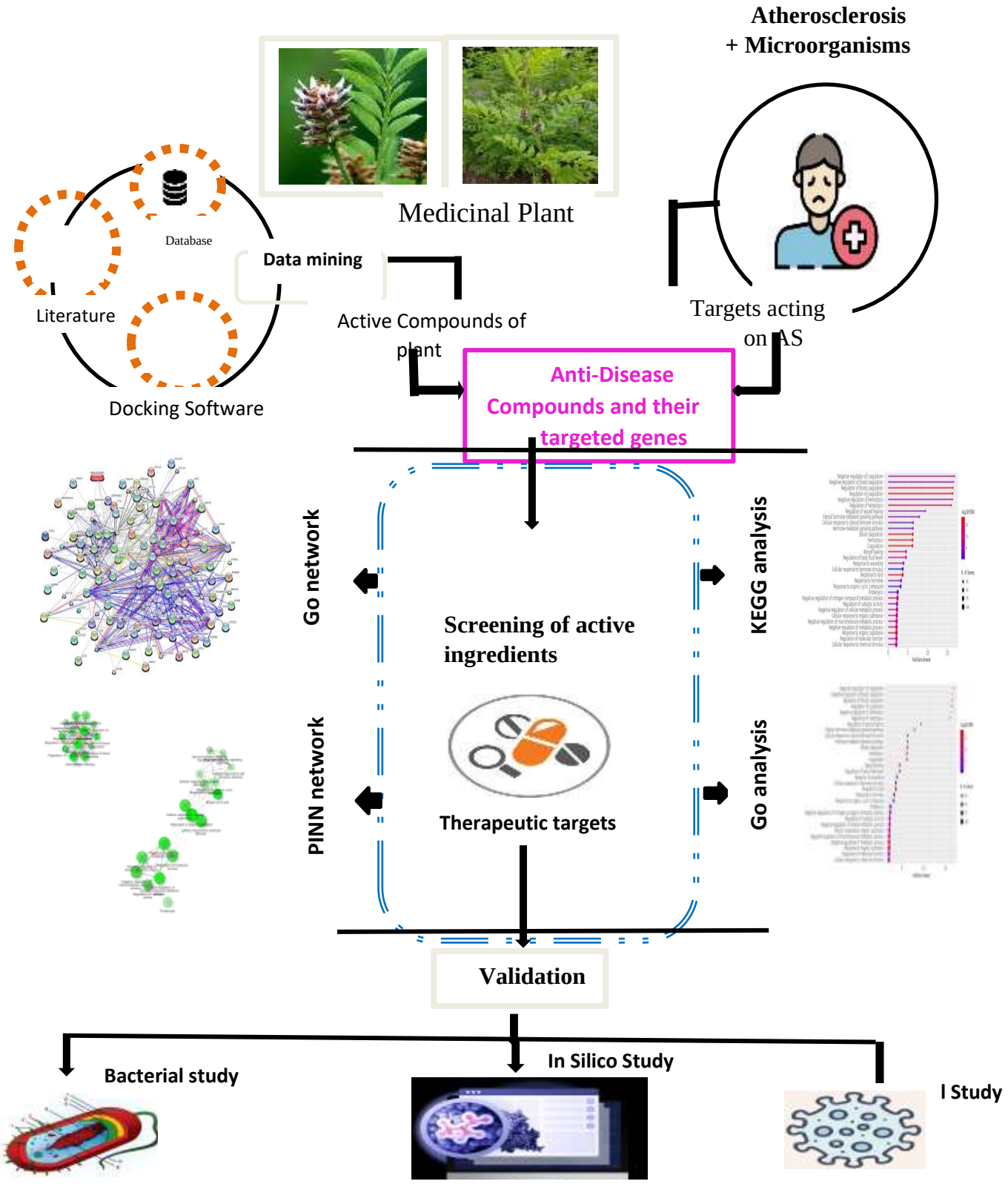

Fig:2 Graphical synopsis of network pharmacology research for the discovery of herbal medicine.

Investigation of Enrichment and Gene Ontology:

An international consensus system for classifying genes according to their functions is called Gene Ontology (GO). Genomic information and pathway annotations are linked by the Kyoto Encyclopedia of Genes and Genomes (KEGG), a knowledge base for systematic gene function analysis. Functional enrichment analyses such as GO and KEGG are widely used to identify potential biological functions of genes (Zhihong Huang et al.,2022). The features of the biological properties of the prospective attacked had been discovered or assessed by structurally and hierarchically analyzing target genes using GO enrichment analysis, based on biological words. A Gene Ontology Enrichment research was conducted using Shiny GO v. 0.741

(http://bioinformatics.sdstate.edu/go74/) to predict the molecular mechanisms of licorice in the treatment of AS. (Hafiza Ayesha Andleeb et al., 2022). The outcomes of the GO biological process and the KEGG pathway enrichment analysis were saved and each term's P values adjusted for the false discovery rate.

Active ingredient and core target docking simulation:

CB-Dock is a docking technique for ligands and proteins that finds binding sites on its own. The molecular docking technique, which informs us about the remodeling of molecules and the interaction of small protein molecules on the large binding sites, is used to predict the binding affinity between two molecules. We used CB-Dock to process ligands and receptors, dock molecules, and examine the outcomes of that docking. The energy of the ligand and receptor molecules must be reduced before the molecular docking process can begin. Additionally, magnetic field and charge must be added, polar hydrogen atoms must be added, and water molecules from small acceptor molecules (PDB files) must be removed. The data was displayed using Discovery Studio, and the hydrogen bonds and the places where they were bound were examined. Through a mathematical function of the receptor and ligand's binding potential, docking energy can be determined. This in-silico work involved calculating the binding capacity of a chemical with its potential binding site using the free binding energies of both. Higher affinities of the molecules and free binding energy have an inverse relationship when it comes to successful docking (Hafiza Ayesha Andleeb et al., 2023)

Results:

Potential licorice and AS targets:

The information of compounds and targets was gathered and checked out in a database for network pharmacology analysis. Tables 1 and 2 provide information on 125 licorice compounds that have 39 target genes. The findings demonstrated that the pharmacological profile of licorice was involved in a target genes and wide range of compounds. Similar to this, several targeted genes were connected to the AS pathology.

Table:2. Ingredients and some properties of licorice screened from database

Mol ID	Molecule Name	MW	OB (%)	DL
MOL002311	Glycyrol	366.39	90.78	0.67
MOL000263	oleanolic acid	456.78	29.02	0.76
MOL000359	sitosterol	414.79	36.91	0.75
MOL000422	kaempferol	286.25	41.88	0.24
MOL004328	naringenin	272.27	59.29	0.21
MOL000467	Castanin	298.31	23.54	0.27

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Network PPI

A network of PPI should be conducted and examined in order to learn more about how overlapping targets interact with one another. 39 overlapping proteins were offered to STITCH so that it could build a PPI network, as seen in Fig. 3. It was composed of the following association of 39 protein targets, 51 edges, and 13 nodes, with an average network probability value of 0.400. Ten functional interactions made up the PPIN end point. The created network's specifics show that its p-value is 1.49e-06. Small p-value is denoted by the significant variety of edges in a network and the non-random selection. The order values and cluster coefficient in this PPIN are 0.836,

respectively. Here, we can see that EGFR's inflammatory process is at its most extreme. Other proteins like UBC, HGS, and GRB2 are then present. It demonstrates the wide range of capabilities this protein possesses, including activation, inhibition, binding, catalysis, modification following translation, and expression.

Table:3. Protein Targets of licorice ingredients associated with Atherosclerosis

1167	Interstitial collagenase	Myocardial infarction (MI)
1198	Serum paraoxonase/arylesterase 1	Cardiovascular disease, unspecified
1233	Cathepsin B	Ischemia
136	Estrogen receptor	Coronary atherosclerosis
136	Estrogen receptor	Cardiovascular disease, unspecified
136	Estrogen receptor	Myocardial Infarction
136	Estrogen receptor	Cardiovascular disease
1502	Peroxisome proliferator activated receptor delta	Atherosclerosis
1502	Peroxisome proliferator activated receptor delta	Hyperlipidemia
1629	Transcription factor AP-1	Vascular disease
1649	Prostaglandin G/H synthase 2	Acute coronary syndromes
1649	Retinoic acid receptor RXR-beta	Atherosclerosis
1721	Tyrosine-protein kinase JAK2	Ischemia
1757	Thrombin	Atherosclerosis

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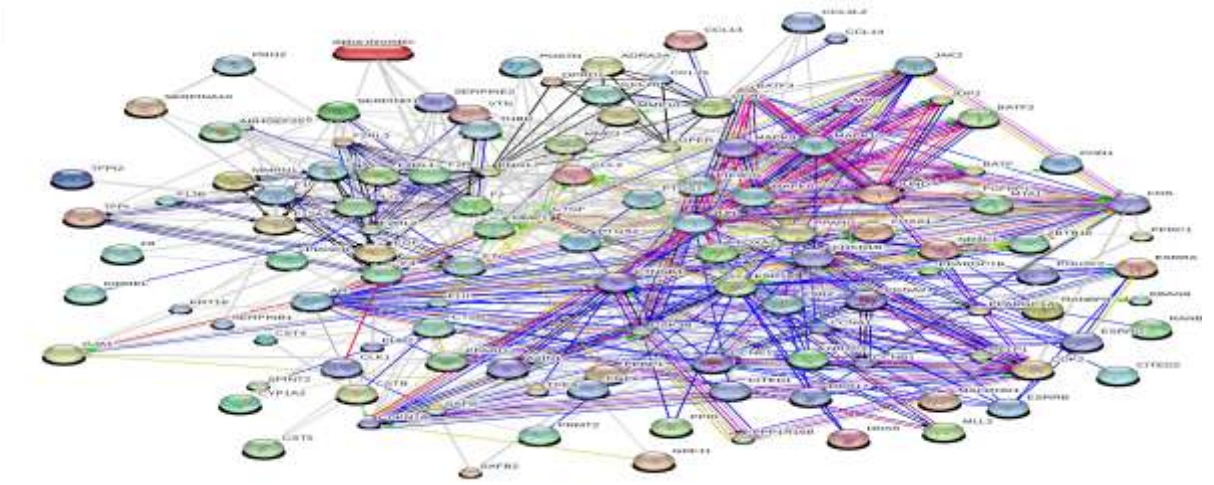

Fig: 3. Network of PPIN demonstrated the interaction of overlapping target proteins between licorice and AS.

Table.4: Node degree of *licorice* targets obtained via STITCH.

Nodes	Node Degree
ESRRA	11
WDR54	2
SPATA20	0
RANBP9	8
FAM136A	0
LMCD1	0
KRT20	0
FGF20	10

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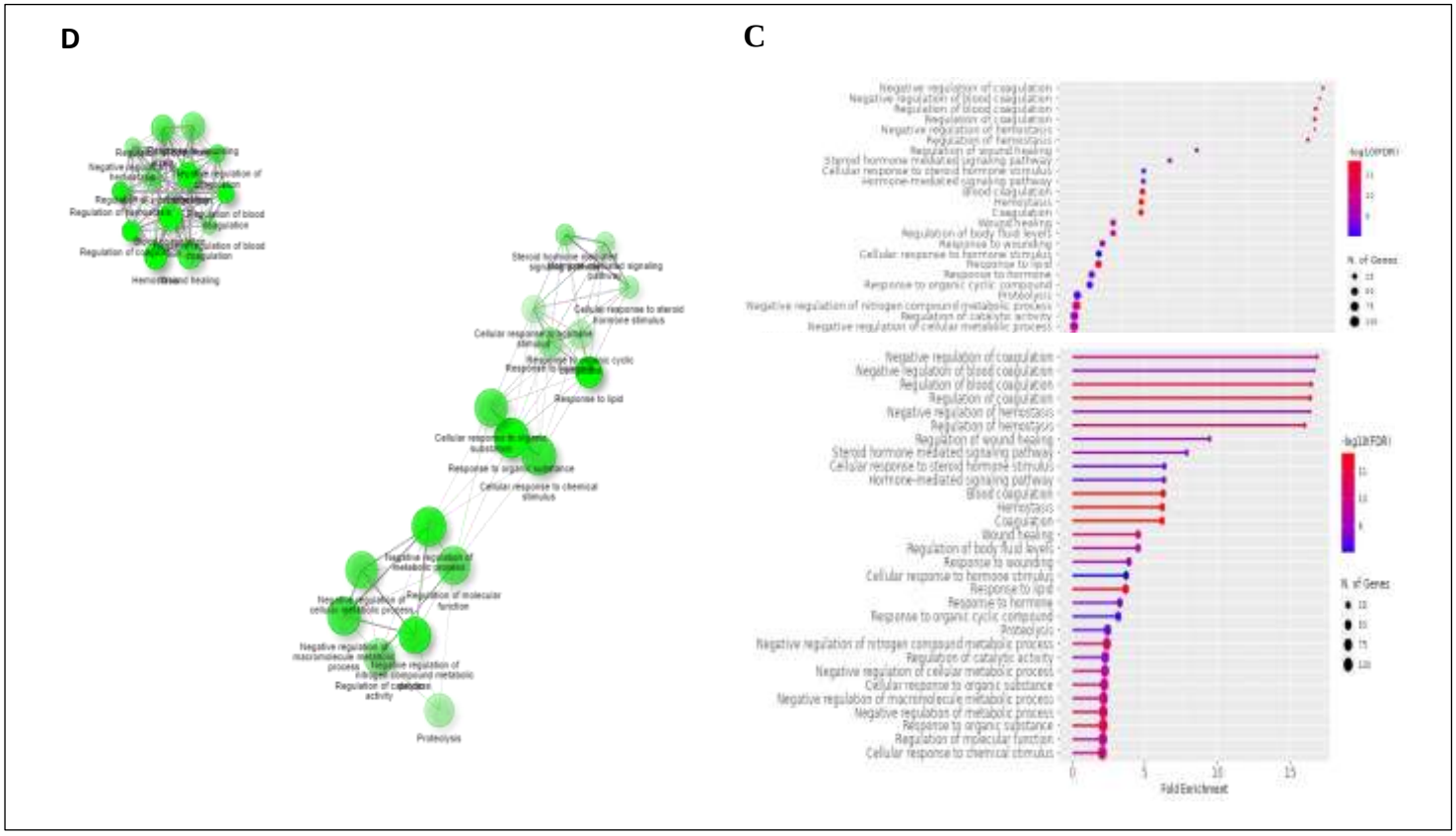

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(c). Dot and Lolli plot colour and size of the dots represent the regulation and the number of DEGs mapped to the indicated pathways, respectively (D). Enriched GO molecular component terms visualized as a network

Table 5. GO terms and their related genes obtained through Shiny GO

Enrichment FDR	nGenes	Pathway Genes	Fold Enrichment	Pathway	Genes
2.29E-12	30	373	6.257537355	Blood coagulation	TFPI F7 JAK2 SERPIND1 PROCR TFPI2 PIK3CG ALOX12 VTN KNG1 F3 PLG F13A1 FOXA2 F10 PROZ F2RL3 SERPINE2 MMRN1 F13B DGKQ ADRA2A F2RL2 F2RL1 THBD F2 F2R F8 F5 SERPINA10

2.29E-12	30	377	6.191144386	Hemostasis	TFPI F7 JAK2 SERPIND1 PROCR TFPI2 PIK3CG ALOX12 VTN KNG1 F3 PLG F13A1 FOXA2 F10 PROZ F2RL3 SERPINE2 MMRN1 F13B DGKQ ADRA2A F2RL2 F2RL1 THBD F2 F2R F8 F5 SERPINA10
2.29E-12	30	378	6.174765697	Coagulation	TFPI F7 JAK2 SERPIND1 PROCR TFPI2 PIK3CG ALOX12 VTN KNG1 F3 PLG F13A1 FOXA2 F10 PROZ F2RL3 SERPINE2 MMRN1 F13B DGKQ ADRA2A F2RL2 F2RL1 THBD F2 F2R F8 F5 SERPINA10
2.29E-12	16	76	16.37937848	Regulation of coagulation	TFPI F7 PROCR ALOX12 VTN KNG1 F3 PLG FOXA2 F10 SERPINE2 F2RL1 THBD F2 F2R F8
4.65E-12	48	1017	3.672073052	Response to lipid	TFPI MPO PON1 IFT88 F7 PTGS2 HSD17B2 ESR1 JAK2 DDX17 ACP5 ABCA2
					MAPK8 DDX5 CCL2 ALOX12 PPARGC1A PPARD NR3C1 ERRFI1 ESRRB DDX54 CDKN1A CITED1 FOXA1 SAFB2 JUND CCDC62 PPARG POSTN ESR2 CYP1A2 GJA1 PPARGC1B PRMT2 SAFB AR FOS MYD88 ESRRA ACER2 JUN THBD F2R AKR1C1 ESRRG PHB2 RXRB

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Table:6. Molecular docking results of key active ingredients and core targets

Number	Protein	PDB ID	Test Compound	Affinity (Kcal/mol)
1	Estrogen receptor	1A52	Glycerol	-7.9
2	Transcription factor AP-1	1A02	Oleanolic Acid	-8.9
3	Prostaglandin G/H synthase 2	1CX2	Sitosterol Kaempferol Naringenin Castanin	-10.3 -8.7 -8.8 -9
4	Thrombin	4BOH	Sitosterol Kaempferol Naringenin Castanin	-8.9 -8.8 -7.7 -8
5	Tyrosine-protein Kinase JAK2	2B7A	Sitosterol Kaempferol Naringenin Castanin	-9.9 -9.1 -9.1 -7
6	Retinoic acid receptor RXR-beta	1H9U	Sitosterol Kaempferol Naringenin Castanin	-9.5 -8.3 -8.7 -9.3

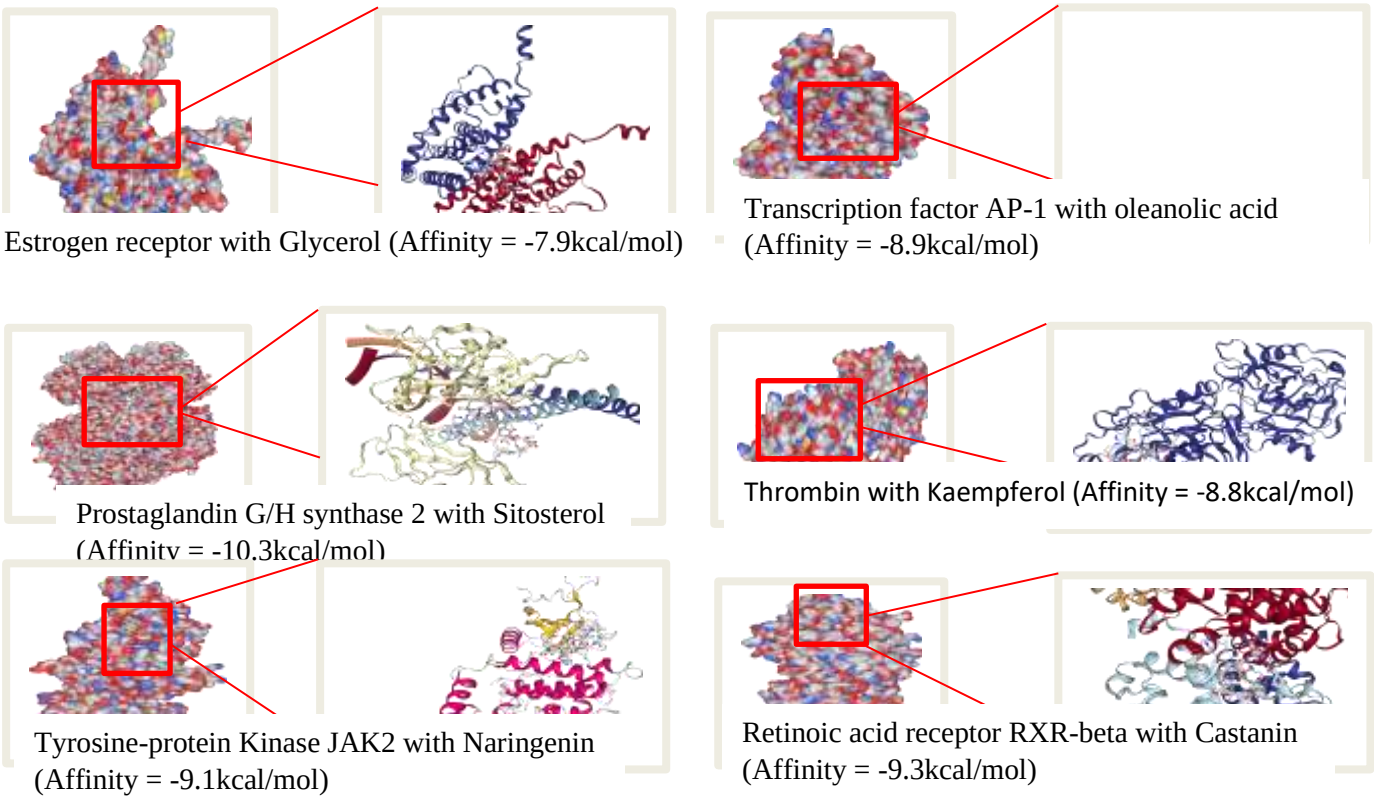

Fig:5. Molecular docking diagram of core targets complexed with key active ingredients.

Discussion:

In order to address the serious public health problem of CAD in China, some researchers have produced treatment manuals. (Sida Jia et al.,2020). Currently, anti-inflammatory, antiapoptotic, anti-atherosclerotic, antiangiogenic, and antithrombogenic medications are the mainstays of treatment for CAD. Today, atherosclerosis is viewed as a chronic inflammatory condition. Additionally, it has been proposed that Particular immune response and infection with C. pneumoniae are related. Studies on men with coronary heart disease suggest that C. pneumoniae may contribute to the development of coronary atherosclerosis through cell-mediated responses that are specific to chlamydia and are primarily triggered by antigenic structures that are shared by various chlamydial species. Another theory suggests that long-term H. pylori infection may alter the serum lipid profile, raising the risk of AS. Constans et al. have demonstrated that Paton et al. discovered major atherosclerosis in coronary arteries in the absence of an associated cardiovascular risk factor during postmortem examination of eight HIV-positive male patients. CMV infection has the potential to infect smooth muscle cells and blood vessel endothelium according to in vitro research, causing endothelium damage and suggesting a role in atherosclerosis. A human atherosclerosis pathogenic role for latent HSV infection of vascular cells has been suggested by an in vitro study using HUVEC cells (cultured primary endothelial cells from the human umbilical vein). A measles virus infection may increase the risk of developing atherosclerosis by damaging endothelial cells and causing the proliferation of smooth muscle cells, according to an in vitro study in isolated endothelium/smooth muscle cells (S A Morré et al.,2001)

TCM is frequently used to treat illnesses and has a long history of use in clinical practice; many of its resources also merit further study. (Jung Chao et al., 2017). By concentrating on specific cell signaling nodes among the complex ingredients found in herbal medicines, unique combinations in traditional complex formulas may be crucial in treating some chronic diseases (Frank R. Stermitz et al., 2000). Network pharmacology has recently been used to examine how medications work to treat disease. It methodically reveals the therapeutic effects of drugs in diseases based on interactions between drugs, targets, and diseases (Wei Zhou et al., 2020). In order to better comprehend the potential mechanisms, network pharmacology and molecular docking analyses were used to pinpoint the underlying molecular mechanisms of licorice's bioactive compounds.

According to the research on network pharmacology, the active components of licorice are more likely to affect the following protein targets in the body, estrogen receptor, prostaglandin G/H synthase 2, thrombin, tyrosine-protein Kinase JAK2, and retinoic acid receptor RXR-beta. Estrogen is thought to protect coronary arteries from atherosclerosis, and part of this protection appears to be mediated by improved serum lipid profiles (D W Losordo et al.,19914). A significant increase in PGHS-2, but not PGHS-1, was seen in cells treated with 25OHC and IL-1 beta, according to a Western immunoblot analysis using anti-PGHS-1 and -2 antibodies on cell lysates. Understanding the role that oxysterols play in the pathogenesis of atherosclerosis by modulating the production of vascular eicosanoids through enzyme induction is possible.

(E R Wohlfeil et al., 1997). Glutathione S-transferase experiments demonstrate the physical interactions of c-Jun, p65, and CBP with PPARalpha. Overall, these results indicate that fibrates suppress PPARalpha-mediated vascular inflammatory responses by inhibiting the ability of NF-kappaB and AP-1 to transactivate via direct protein-protein associations with p65 and c-Jun. (P Delerive et al., 1999). The pathophysiology of vascular calcification and the development of atherosclerosis is influenced by thrombin. Antithrombotic therapy is an essential component of the treatment and prevention of atherothrombosis in patients (Julian I. Borissoff MD et al., 2012).

In this article, there is analysis and discussion about basic mechanism of action of naringin and naringinin in the vascular and cardiovascular diseases (Reza Heidary Moghaddam et al., 2020). A flavonoid called kaempferol that is present in many plants has proven to have cardioprotective effects in numerous cardiac injury models. Myocardial fibrosis, oxidative stress, and inflammation are decreased, while mitochondrial activity and calcium homeostasis are preserved, kaempferol enhances cardiac function (Yusof Kamisah et al., 2022). Oleanolic acid affected serum lipid levels, lipid buildup in the liver, and intimal artery thickening, all of which slowed the progression of atherosclerosis. And the lipid metabolism genes PPAR, AdipoR1, and the underlying mechanism of OA on atherosclerosis may involve adipoR2. (Hanqiong Luo et al., 2017). Several clinical and preclinical investigations indicate that -sitosterol has a wide range of important health advantages. It decreases levels of

harmful cholesterol (LDL), lowers the risk of coronary artery disease, heart attacks, and atherosclerosis, prevents many cancers, and supports the body's natural healing process (Ena Gupta et al., 2020).

In conclusion, it's unclear how AS develops. The signal transduction exhibits a multi-target and multi-pathway pattern due to cross-linking and mutual modulation. Licorice-derived chemicals alter target genes and proteins that are crucial for maintaining signal pathways. The outcomes demonstrated that TCM acts in a multi-compound, multi-target, multi-pathway manner. It is still important to carry out additional research on the signaling pathways and targets affected by the pharmacological properties of licorice on AS.

Conclusion:

As previously mentioned, a network pharmacology approach was suggested for inquiry into the regulatory pathways, active ingredients, and AS treatment-related active ingredients of licorice. The findings identified the chemical components that give licorice its anti-inflammatory and anti-thrombogenic properties, as well as the underlying molecular mechanisms. Previous research has partially supported these results. The key active ingredients of licorice, Castanin, Naringenin, Kaempferol, Sitosterol, Glycerol, and Oleanolic Acid, each had a favourable binding affinity for the primary target particles, supporting the potential molecular mechanisms of licorice in AS. This investigation provides a comprehensive resource for studying the therapeutic mechanisms of licorice in AS.

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