

Mechanism of Action of Agarwood Essential Oil in Enhancing Myocardial Ischemia: Insights from Network Pharmacology and Experimental Validation

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Abstract [Objectives] To investigate the protective effects and underlying mechanisms of agarwood essential oil against isoprenaline (ISO)-induced myocardial ischemia (MI) in mice. [Methods] Utilizing network pharmacology methods, the active components, targets, and MI-related targets of agarwood were identified. Subsequently, Gene Ontology (GO) functional analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were performed on the identified targets. The efficacy and predictive pathways were evaluated using MI mice. Thirty male C57 mice were randomly divided to five groups: the control group (CTL), the model group (MOD), the positive control group (Pro), the low-dose agarwood essential oil group (Y01-L), and the high-dose agarwood essential oil group (Y01-H). Mice in each treatment group received continuous intragastric administration for 14 d. Beginning on day 8, 1 h post administration, mice in all groups except the control group were intraperitoneally injected with ISO at a dose of 10 mL/kg daily for 7 d. The control group received an equivalent volume of normal saline via intraperitoneal injection during this period. One hour following the final administration, blood samples were collected under anesthesia, and the heart was excised and weighed to determine the organ index. The activities of lactate dehydrogenase (LDH) and superoxide dismutase (SOD), and the content of malondialdehyde (MDA) in mouse serum were measured using biochemical assay kits. The protein expression levels of HIF-1 α , P-AKT, and P-PI3K were analyzed using Western blot assay. [Results] The results of the network pharmacology analysis identified AKT1, MAPK14, and other targets as common to both agarwood and MI. GO functional analysis and KEGG pathway enrichment analysis demonstrated a significant association with the HIF-1 α signaling pathway. The validation results in mice demonstrated that the active component, agarwood essential oil Y01, effectively inhibited cardiac swelling induced by ischemia. Serological indicators revealed that, compared to the model group, Y01 dose-dependently decreased serum MDA level and LDH activity ($P < 0.001$) while increasing SOD activity ($P < 0.01$, $P < 0.001$). The Western blot assay demonstrated that Y01 significantly upregulated the protein expression levels of HIF-1 α , P-AKT, and P-PI3K. [Conclusions] Y01 has the potential to mitigate myocardial injury induced by ischemia through the modulation of the HIF-1 α and AKT/PI3K signaling pathways, thereby enhancing cardiac function.

Key words Myocardial ischemia, Agarwood essential oil, Network pharmacology, HIF-1 α

1 Introduction

Ischemic heart disease (IHD) exhibits a high incidence and mortality rate both in China and globally, posing a significant threat to human health and property^[1]. Myocardial ischemia (MI) is the primary cause of IHD. A reduction in blood perfusion leads to decreased oxygen supply to the brain, resulting in abnormal myocardial energy metabolism. Prolonged ischemia can cause a loss of myocardial contractility, which may induce irreversible myocardial damage. Clinically, this condition typically presents as myocardial infarction. Currently, antiplatelet drugs (such as aspirin), β -blockers (such as metoprolol), nitrate drugs (such as nitroglycerin), and statins (such as atorvastatin) are commonly employed in clinical practice. These medications serve to reduce thrombosis, decrease cardiac workload, dilate blood vessels, and stabilize atherosclerotic plaques. However, prolonged use may elevate the risk of bleeding and induce adverse effects such as hypotension and bradycardia^[2–3].

According to its etiology and pathogenesis, traditional

Chinese medicine classifies IHD under the categories of thoracic obstruction, cardiodynia, and angina pectoris, and possesses extensive clinical experience in the prevention and treatment of these conditions. Thoracic obstruction is characterized as a syndrome involving deficiency at its root and excess at its superficial level. This condition is primarily attributed to spleen deficiency and dysfunction of the spleen and stomach, with clinical manifestations including phlegm turbidity, blood stasis, qi stagnation, cold coagulation, and other tangible pathogenic factors that obstruct the heart meridian. Agarwood is recognized as one of the top ten "southern medicinal substances" and is derived from the resinous wood of *Aquilaria sinensis* (Lour.) Gilg, a species belonging to the family Agaraceae. According to the 2020 edition of the *Pharmacopoeia of Traditional Chinese Medicine*, agarwood possesses a pungent and bitter taste and exhibits a slightly warm nature. It is traditionally attributed with functions such as promoting qi circulation and alleviating pain, warming the middle energizer to prevent vomiting, and facilitating inspiration to relieve asthma. Clinically, it is employed in the treatment of chest and abdominal distension and pain, vomiting and hiccups caused by cold in the stomach, shortness of breath associated with kidney deficiency and reversed flow of qi, etc.^[4]. It is extensively utilized as a primary ingredient in traditional Chinese medicine formulations for the treatment of cardiovascular-related diseases, including Bawei Chenxiang powder, Bawei Qingxin Chenxiang powder, and Zaizao pills^[5]. Agarwood comprises various solid condensates, including gum, res-

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in, essential oil, and wood. Among these components, agarwood essential oil serves as the primary active component group and aromatic substance, possessing considerable research and development potential^[6]. However, the mechanism underlying its efficacy in improving MI remains inadequately understood. Therefore, this study predicted the mechanism of action of agarwood in improving MI injury using network pharmacology and validated these findings through an MI mouse model induced by isoprenaline (ISO), in order to provide a theoretical foundation for its clinical application.

2 Materials and methods

2.1 Experimental animals Thirty SPF male C57BL/6 mice, weighing 18–20 g, were obtained from the Guangdong Medical Laboratory Animal Center (Certificate No.: SCXK [Y] 2022-0002; Animal Quality Certificate No.: 44007200114336). During the experiment, the mice were housed in the barrier animal laboratory of Zhongshan Hospital of Traditional Chinese Medicine (License No.: SYXK [Y] 2020-0109). The environment was controlled with a 12 h light/dark cycle, at a temperature of (22 ± 2) °C and relative humidity between 50% and 60%. The mice had *ad libitum* access to food and water. Following a 3 d acclimation period, the experiment was initiated. The animal experiments were reviewed and approved by the Ethics Committee of Zhongshan Hospital of Traditional Chinese Medicine (Approval No.: AEW-2024077). All procedures were conducted in strict accordance with the relevant ethical regulations governing animal experimentation.

2.2 Drugs and reagents Agarwood essential oil (Y01) was produced by Zhongshan Yuanyi Agarwood Industry Investment Co., Ltd. Isoprenaline (ISO) was obtained from Sigma-Aldrich (Shanghai) Trading Co., Ltd. (Item No.: 002Q67), and propranolol (Pro) was supplied by Jiangsu Yabang Aipusen Pharmaceutical Co., Ltd. (Item No.: E221017). The SOD detection kit (Item No.: A001-3-2) and LDH detection kit (Item No.: A020-2-2) were purchased from Nanjing Jiancheng Bioengineering Institute. The MDA detection kit (Item No.: S0131S), RIPA lysate (Item No.: P0013B), protease and phosphatase inhibitor mixture (Item No.: P1046), and primary antibody dilution reagent (Item No.: P0256-500 mL) were acquired from Beyotime Biotechnology Company. The BCA protein quantification kit (Item No.: 23225) was procured from Thermo Fisher Scientific (China) Co., Ltd. β -Actin (Item No.: AF7018), P-PI3K (Item No.: AF3241), P-AKT1 (Item No.: AF0016), HIF-1 α (Item No.: AF1009), and Super ECL (Item No.: #2120b02) were all obtained from Jiangsu Affinity Biosciences Co., Ltd. The PVDF membrane (0.45 μ m) was produced by Millipore China Co., Ltd.

2.3 Instrument The AUW120D electronic balance was manufactured by Shimadzu Enterprise Management (China) Co., Ltd.; the 5910Ri high-speed refrigerated centrifuge was produced by Eppendorf; the LUKYM-1 sample cryogenic grinder was made by Guangzhou Luka Sequencing Instrument Co., Ltd.; the VICTOR Nivo multifunctional microplate reader was supplied by Thermo Fisher Scientific (China) Co., Ltd.; and the EPHC 400 dual independent high-current electrophoresis power supply was manufactured by Guangzhou Daoyi Science and Technology Co., Ltd.

2.4 Prediction of drug-target-disease via network pharmacology

2.4.1 Collection of components and targets of agarwood. The chemical components of agarwood were obtained from the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) (<https://old.tcmsp-e.com/tcmsp.php>). Screening criteria for these chemical components included an oral bioavailability (*OB*) of at least 30% and a drug-likeness (*DL*) coefficient of at least 0.18. The SDF chemical structures of the screened chemical components were retrieved from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>). The corresponding targets of these components were identified using the Swiss Target Prediction database (<http://www.swisstargetprediction.ch/>), with the species condition set to "Homo sapiens". The target information was imported into the TCMSP database to obtain the molecular number.

2.4.2 Collection of key component targets of diseases. MI-related targets were retrieved from GeneCards (<https://www.genecards.org/>) and subsequently filtered based on a relevance score greater than 9.

2.4.3 Construction of drug-disease interaction network. Drug component targets and disease-related targets were imported into Venny 2.1 (<https://bioinfogp.cnb.csic.es/tools/venny/index.html>) to generate a Venn diagram and identify overlapping targets. These overlapping targets were the potential therapeutic targets of agarwood in the treatment of MI. Subsequently, the intersection targets were imported into Cytoscape 3.9.1 software to construct a drug-disease interaction network.

2.4.4 Construction of protein-protein interaction (PPI) network. The identified intersection targets were imported into the STRING database (<http://cn.string-db.org/>), with the species specified as "Homo sapiens". A protein-protein interaction score threshold greater than 0.7 was applied, and the resulting PPI network diagram was generated to elucidate the relationships among potential target proteins.

2.4.5 GO and KEGG enrichment analysis. The intersection targets were imported into the Metascape database (<https://metascape.org/>) for GO functional analysis and KEGG pathway enrichment analysis. The species was specified as "Homo sapiens". Analyses were conducted for biological processes (BP), cellular components (CC), molecular functions (MF), and metabolic pathways, with the top ten results from each category subsequently visualized.

2.5 Animal grouping and modeling for drug administration

Thirty C57BL/6 mice were randomly divided to 5 groups: the control group (CTL), the model group (MOD), the propranolol group (Pro, 30 mg/kg), the low-dose agarwood essential oil group (Y01-L, 0.25 mL/kg), and the high-dose agarwood essential oil group (Y01-H, 0.5 mL/kg), with 6 mice per group. Mice in each group had free access to food and water. Except for the control and model groups, the remaining groups received intragastric administration of the drug at predetermined doses for 2 weeks. Beginning on day 8, all groups, except the control group which was injected with an equivalent volume of normal saline, were intraperitoneally injected with ISO at a concentration of 10 mg/kg

daily for consecutive 7 d^[7]. One hour following the final administration, the sacrificed mice were anesthetized. Blood and heart samples were subsequently collected. The heart was weighed, bisected vertically, with one half fixed in 4% paraformaldehyde and the other half preserved in liquid nitrogen.

2.6 Cardiac index analysis Following dissection, the morphology and dimensions of the heart were documented, and its weight was measured. The organ index was calculated using the following formula: Cardiac index = (Cardiac weight/Body weight of mouse) × 100%.

2.7 Detection of serum indicators in mice The mouse plasma was allowed to stand for 2 h, followed by centrifugation at 4 000 rpm for 10 min at 4 °C. The supernatant was collected, and the activities of superoxide dismutase (SOD) and lactate dehydrogenase (LDH), as well as the malondialdehyde (MDA) content, were measured according to the procedures outlined in the kit manual.

2.8 Western blot assay The cardiac tissue was weighed and homogenized in pre-cooled RIPA lysis buffer containing protease and phosphatase inhibitors at a ratio of 1 : 10. Following homogenization, the mixture was centrifuged at 12 000 rpm for 15 min, and the supernatant was collected. Protein concentration was determined using the BCA assay, after which the samples were mixed with loading buffer and heated to induce denaturation. Equal amounts of protein samples were separated by electrophoresis under constant pressure using a 10% polyacrylamide gel (SDS-PAGE) and subsequently transferred to a PVDF membrane. The membrane was blocked with 5% skimmed milk powder for 2 h.

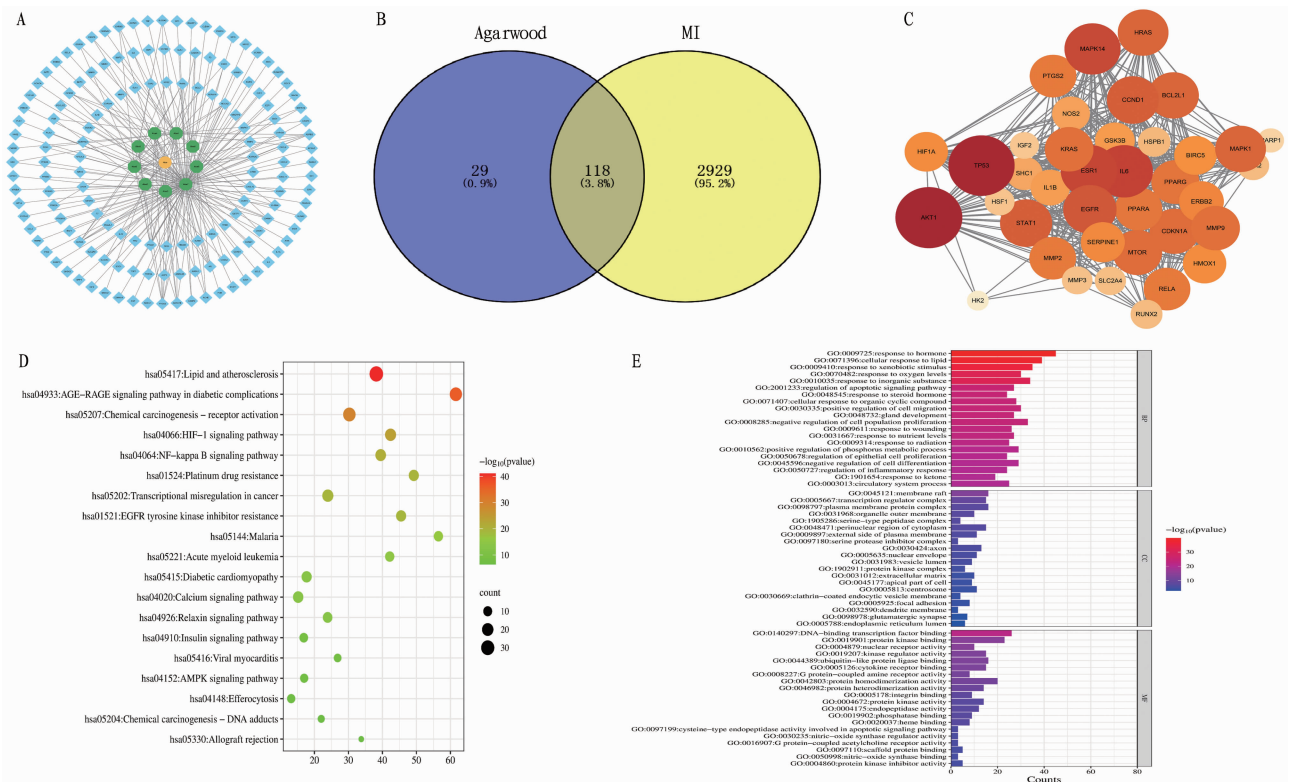
Following washing with PBST, the membrane was incubated overnight at 4 °C with primary antibodies against HIF-1 α , P-AKT, and P-PI3K. The following day, the film was washed with PBST, after which the rabbit secondary antibody, diluted at a ratio of 1 : 10 000, was applied and incubated for 2 h. Subsequently, the film was washed again with PBST, and the developing solution was added. Imaging was conducted using a chemiluminescence imaging system.

2.9 Statistical analysis All obtained results were analyzed using SPSS 24.0 software and visualized with GraphPad Prism 7.00. Statistical significance was determined at a threshold of $P < 0.05$.

3 Results and analysis

3.1 Target information predicted by network pharmacology

A total of 9 active components of agarwood were identified through database analysis, including beta-sitosterol (Aloe1), sitosterol (Aloe2), boldine (Aloe3), norboldine (Aloe4), DMPEC (Aloe5), 6,7-dimethoxy-2-(2-phenylethyl) chromone (Aloe6), quercetin (Aloe7), C09495 (Aloe8), and nubigenol (Aloe9). Subsequently, 147 targets were screened (Fig. 1A). Through database screening, a total of 3 047 disease-related targets were identified, including 118 overlapping targets associated with agarwood, such as AKT1 and MAPK14 (Fig. 1B-C). The results of the KEGG pathway analysis indicated that agarwood may improve myocardial injury induced by ischemia through modulation of the HIF-1 α signaling pathway.



NOTE A. Effective components of agarwood-target network diagram; B. Venn diagram of drug-disease intersection targets; C. PPI network diagram; D. KEGG pathway enrichment analysis; E. GO functional enrichment analysis.

Fig. 1 Target information of agarwood in the treatment of MI via network pharmacology

3.2 Protective effect of agarwood essential oil on the heart of MI mice Compared to the control group, the hearts of mice in the model group exhibited marked hypertrophic changes, accompanied by a significant increase in the cardiac index. In contrast, mice treated with Y01 demonstrated a gradual normalization of cardiac morphology, with no evident enlargement, and a significant reduction in the cardiac index (Table 1).

Serological analysis demonstrated that, relative to the control

group, mice in the model group exhibited a significant increase in serum LDH activity ($P < 0.001$), a significant decrease in SOD activity ($P < 0.001$), and a significant elevation in MDA content ($P < 0.001$). In the Y01 treatment group, mice exhibited a significant decrease in LDH activity ($P < 0.001$) and MDA content ($P < 0.001$), alongside a significant increase in SOD activity ($P < 0.001$), indicating a dose-dependent effect.

Table 1 Effect of agarwood essential oil on cardiac index in MI mice ($\bar{x} \pm s$, $n = 6$)

Group	Dose	Cardiac index // %	MDA // $\mu\text{M}/\mu\text{g}$	SOD // U/mL	LDH // U/L
CTL		5.66 $\pm$ 0.50	6.27 $\pm$ 1.87	29.53 $\pm$ 3.43	6 377.86 $\pm$ 346.96
MOD		6.57 $\pm$ 0.63 ^{##}	41.72 $\pm$ 3.95 ^{###}	24.19 $\pm$ 2.66 ^{###}	11 514.12 $\pm$ 775.13 ^{###}
Pro	30.00 mg/kg	5.68 $\pm$ 0.42 ^{**}	31.27 $\pm$ 2.83 ^{**}	35.98 $\pm$ 3.94 ^{***}	6 368.64 $\pm$ 510.39 ^{***}
Y01-L	0.25 mL/kg	5.77 $\pm$ 0.29 ^{**}	22.39 $\pm$ 3.86 ^{***}	33.47 $\pm$ 2.35 ^{***}	6 538.95 $\pm$ 970.32 ^{***}
Y01-H	0.50 mL/kg	5.83 $\pm$ 0.39 ^{**}	13.94 $\pm$ 1.91 ^{***}	37.30 $\pm$ 2.38 ^{***}	5 929.18 $\pm$ 856.33 ^{***}

NOTE Compared to the CTL group, ^{##} $P < 0.01$, ^{###} $P < 0.001$; Compared to the MOD group, ^{**} $P < 0.01$, ^{***} $P < 0.001$.

3.3 Effect of agarwood essential oil on proteins associated with the HIF-1 α signaling pathway The Western blot assay demonstrated that, compared to the CTL group, the protein expression levels of HIF-1 α , P-PI3K, and P-AKT were significantly decreased in the MOD group ($P < 0.001$, Fig. 2). Following intervention with Y01, the expression levels of HIF-1 α , P-PI3K, and P-AKT were significantly increased in a dose-dependent manner.

ponents of agarwood and MI. Furthermore, KEGG pathway enrichment analysis highlighted the HIF-1 α signaling pathway as a critical pathway involved. In experimental validation, Y01 markedly reduced cardiac hypertrophy in ISO-induced MI mice, improved oxidative stress markers by decreasing serum MDA level and LDH activity while enhancing SOD activity, and upregulated the expression of critical proteins within the PI3K/AKT/HIF-1 α signaling pathway. These results offer significant experimental evidence and a theoretical foundation supporting the therapeutic potential of agarwood essential oil in the treatment of MI.

Mitochondrial dysfunction and disturbances in energy metabolism induced by MI constitute the primary factors driving the excessive generation of reactive oxygen species (ROS)^[8]. The overproduction of ROS initiates lipid peroxidation, as evidenced by increased MDA levels, depletes critical antioxidant enzymes such as SOD, and damages cell membrane integrity. These processes lead to elevated LDH release, culminating in myocardial cell death and impaired cardiac function^[9]. This study observed a significant increase in serum MDA level and LDH activity, alongside a marked decrease in SOD activity in the model group of mice, indicating pronounced myocardial oxidative stress injury. Notably, intervention with Y01 resulted in dose-dependent and significant improvements in these oxidative stress markers. Specifically, the high-dose Y01 group demonstrated effects on reducing MDA level and LDH activity and enhancing SOD activity that were comparable to those observed in the positive control group treated with propranolol. The findings are highly consistent with the well-established potent antioxidant and free radical scavenging properties of agarwood and its active components^[10]. This study further confirms that the mitigation of oxidative stress-induced injury is a key mechanism by which Y01 confers protection to ischemic myocardium, thereby offering direct evidence supporting its clinical utility in addressing oxidative damage associated with IHD.

HIF-1 α serves as a central regulatory factor enabling cellular adaptation to hypoxic conditions. In the context of MI, the stability and activation of HIF-1 α exhibit a dual role^[11]. On the one hand, it can induce the expression of pro-survival genes, including

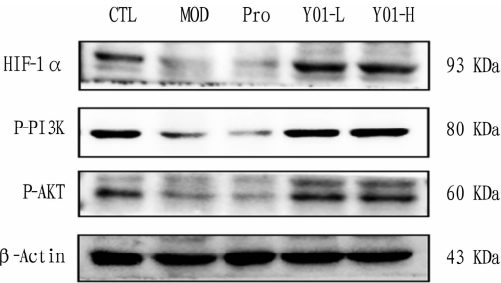


Fig.2 Effect of agarwood essential oil on proteins associated with the HIF-1 α signaling pathway

4 Discussion

IHD is a primary contributor to the ongoing rise in global mortality and disability rates. Therefore, the development of safer and more effective prevention and control strategies is of paramount importance. Agarwood, a traditional Chinese medicinal material, has a long-standing history in the treatment of cardiovascular conditions, including "thoracic obstruction" and "cardiodynia". Its essential oil (Y01), representing a key group of active components, demonstrates considerable potential for research and development. However, the precise molecular mechanism through which Y01 improves MI has not been comprehensively elucidated, thereby limiting its clinical transformation and application. This study integrates network pharmacology predictions with *in vivo* experimental validation to elucidate the mechanism by which Y01 confers cardioprotective effects via modulation of the PI3K/AKT/HIF-1 α signaling pathway. Network pharmacology analysis identified AKT1, MAPK14, and other targets as common to the active com-

