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Research Article

Computational Evaluation of *Nigella sativa* as a Therapeutic Agent in Hyperlipidemia: Network Pharmacology, ADMET, and Molecular Docking Studies

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ABSTRACT

Hyperlipidaemia is a major contributor to cardiovascular diseases and metabolic disorders, often associated with elevated levels of plasma cholesterol and triglycerides. Despite the availability of synthetic lipid-lowering agents, their adverse effects have spurred the search for safer, plant-based alternatives. This study investigates the anti-hyperlipidaemic potential of *Nigella sativa* through an integrative in silico approach encompassing network pharmacology, molecular docking, and ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) analysis. A total of 192 phytoconstituents were identified from *Nigella sativa*, of which three—(E,Z)-Farnesol, Farnesol, and (+)-trans-Piperitenol—showed significant interaction with key hyperlipidemia-related targets: PPARA and PYGL. Network pharmacology analysis revealed 3 overlapping targets between disease-associated genes and compound-predicted targets. Protein-protein interaction analysis identified PPARA and PYGL as key hub genes. Gene ontology and KEGG pathway enrichment analyses highlighted involvement in lipid metabolism, PI3K-Akt signaling, insulin regulation, and inflammatory processes. Molecular docking demonstrated strong binding affinities of the selected phytoconstituents, particularly (E,Z)-Farnesol, which outperformed the reference drug fenofibrate in binding to PYGL. The compounds exhibited favorable pharmacokinetic properties, including high gastrointestinal absorption, blood-brain barrier permeability, and compliance with Lipinski's Rule of Five. Toxicity profiling indicated no hepatotoxic, carcinogenic, or mutagenic risks. Collectively, the findings validate the therapeutic potential of *Nigella sativa* as a multi-targeted, non-toxic natural treatment for hyperlipidemia, offering a promising foundation for future experimental and clinical investigations.

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INTRODUCTION

Cardiovascular diseases (CVDs) remain the leading cause of morbidity and mortality worldwide, with hyperlipidemia recognized as a major modifiable risk factor contributing to their progression. Hyperlipidemia refers to a metabolic condition characterized by abnormal elevations in plasma lipids and lipoproteins, specifically total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C), and triglycerides (TGs), coupled with reduced high-density lipoprotein cholesterol (HDL-C). Such dysregulation of lipid metabolism leads to excessive deposition of cholesterol within arterial walls, promoting atherosclerotic plaque formation and increasing the risk of coronary heart disease (CHD), stroke, and peripheral vascular complications [1]. Several lifestyle factors, including poor dietary patterns rich in saturated fats, sedentary behavior, smoking, excessive alcohol intake, and obesity, contribute significantly to the prevalence of hyperlipidemia. Additionally, genetic predispositions and underlying metabolic disorders such as type 2 diabetes mellitus and hypothyroidism exacerbate lipid imbalance. Notably, hyperlipidemia frequently coexists with obesity, a condition characterized by excessive visceral fat accumulation, further compounding cardiovascular risk. It is well established that individuals with high LDL-C and TGs and low HDL-C are predisposed to the development of coronary artery disease and other metabolic complications. Globally, hyperlipidemia affects millions of adults, with a substantial proportion of cases remaining undiagnosed or inadequately treated. Epidemiological data suggest that nearly 50% of adults in industrialized nations have elevated LDL-C levels, yet less than one-third effectively manage their lipid profiles through lifestyle interventions and pharmacotherapy. This

scenario underscores the need for improved preventive strategies and novel therapeutic approaches to mitigate the burden of hyperlipidemia and its associated health consequences [2]. Current treatment regimens for hyperlipidemia predominantly rely on synthetic lipid-lowering agents such as statins, fibrates, bile acid sequestrants, and cholesterol absorption inhibitors. Although effective, these drugs are often associated with adverse effects including liver toxicity, myopathy, gastrointestinal disturbances, and poor patient compliance. Consequently, there is growing interest in identifying safer, cost-effective, and efficacious alternatives derived from natural sources [3]. Medicinal plants have long served as a valuable source of therapeutic agents in traditional and modern healthcare systems. Among them, *Nigella sativa*, commonly known as black seed or black cumin, holds a prominent place due to its wide spectrum of pharmacological properties. Traditionally used across Asia, the Middle East, and Africa, *Nigella sativa* seeds are renowned for their antioxidant, anti-inflammatory, immunomodulatory, hepatoprotective, and hypolipidemic effects. Modern pharmacological studies have validated these traditional claims, revealing that its bioactive constituents can favorably modulate lipid metabolism, reduce oxidative stress, and lower serum lipid levels [77]. Despite extensive ethnopharmacological use, the precise molecular mechanisms underlying the lipid-lowering effects of *Nigella sativa* remain incompletely understood. In recent years, advances in bioinformatics and computational tools have enabled the application of network pharmacology and molecular docking techniques to explore the multi-target interactions of plant-derived compounds at the systems level. This integrative approach provides valuable insights into the complex interactions between



phytochemicals and biological pathways implicated in disease progression.

❖ Review of literature

Cholesterol, triglycerides, and high-density lipoproteins are important constituents of the lipid fraction of the human body. Cholesterol is an unsaturated alcohol of the steroid family of compounds. It is essential for the normal function of all animal cells and is a fundamental element of their cell membranes. It is also a precursor of various critical substances such as adrenal and gonadal steroid hormones and bile acids. Triglycerides are fatty acid esters of glycerol and represent the main lipid component of dietary fat and fat depots of animals (4). Cholesterol and triglycerides, being non polar lipid substances (insoluble in water), need to be transported in the plasma associated with various lipoprotein particles. Plasma lipoproteins are separated by hydrated density; electrophoretic mobility; size; and their relative content of cholesterol, triglycerides, and protein into five major classes: chylomicrons, very-low-density lipoproteins (VLDL), intermediate-density lipoproteins (IDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL) (5). Since the levels of plasma lipids have a bell-shaped distribution in the general population, the definition of either a high or a low value of these substances has remained an arbitrary statistical decision. High values have been traditionally considered as those in the 90th and 95th percentiles; low values were considered to be those below the 5th percentile (6). The NIH Consensus Conference has recently revised the values concerning cholesterol, however, in view of clear evidence of an increased risk of coronary atherosclerosis in persons falling in the 75th to 90th percentiles. According to this last statement, cholesterol levels below 200 mg/dl are classified as "desirable blood cholesterol," those 200 to 239

mg/dl as "borderline-high blood cholesterol," and those 240 mg/dl and above as high blood cholesterol (7).

❖ Epidemiology

There are over three million adults throughout the United States and Europe that currently have a diagnosis of hyperlipidemia, and that number continues to rise at a drastic pace. Hyperlipidemia is typically a chronic, progressive disease process that demands lifestyle and dietary changes, with the potential need for additional lipid-lowering medications. The degree of hyperlipidaemia is highest in patients with premature coronary artery disease (CAD), defined as CAD arising in males before age 55 to 60 years and females before age 65 years. Under the prior specified circumstances, the incidence of hyperlipidemia is around 75-85%, opposed to roughly 40 to 48% in the control population of comparable age, but without the presence of premature coronary artery disease (8). Estimates are that over 50% of American adults have elevated LDL levels, and it is speculated that under 35% of those patients adequately manage their high LDL levels, clearly depicting an undertreated disease. Per the JAMA Network, "Prevalence of dyslipidaemia was significantly greater among whites than blacks (women, 64.7% vs. 49.5%; and men, 78.4% vs. 56.7%; $P < .001$ for both) and amongst men than women ($P \leq .02$ in every ethnic group)" (9). Intuitively, in countries with lower overall rates of obesity and saturated fat consumption, the prevalence of hyperlipidemia and subsequent coronary artery disease is lower, when contrasted to rates in Europe and throughout the United States.

Lipid Metabolism & Pathways of Lipid Transport

The lipid transport system in plasma has been described as involving two pathways: an



exogenous route for the transport of cholesterol and triglycerides absorbed from dietary fat in the intestine, and an endogenous system through which cholesterol and triglycerides reach the plasma from the liver and other non-intestinal tissues. Exogenous and endogenous fat-transport

pathways are diagrammed. Dietary cholesterol is absorbed through the wall of the intestine and is packaged, along with triglyceride (glycerol ester-linked to three fatty acid chains), in chylomicrons (10).

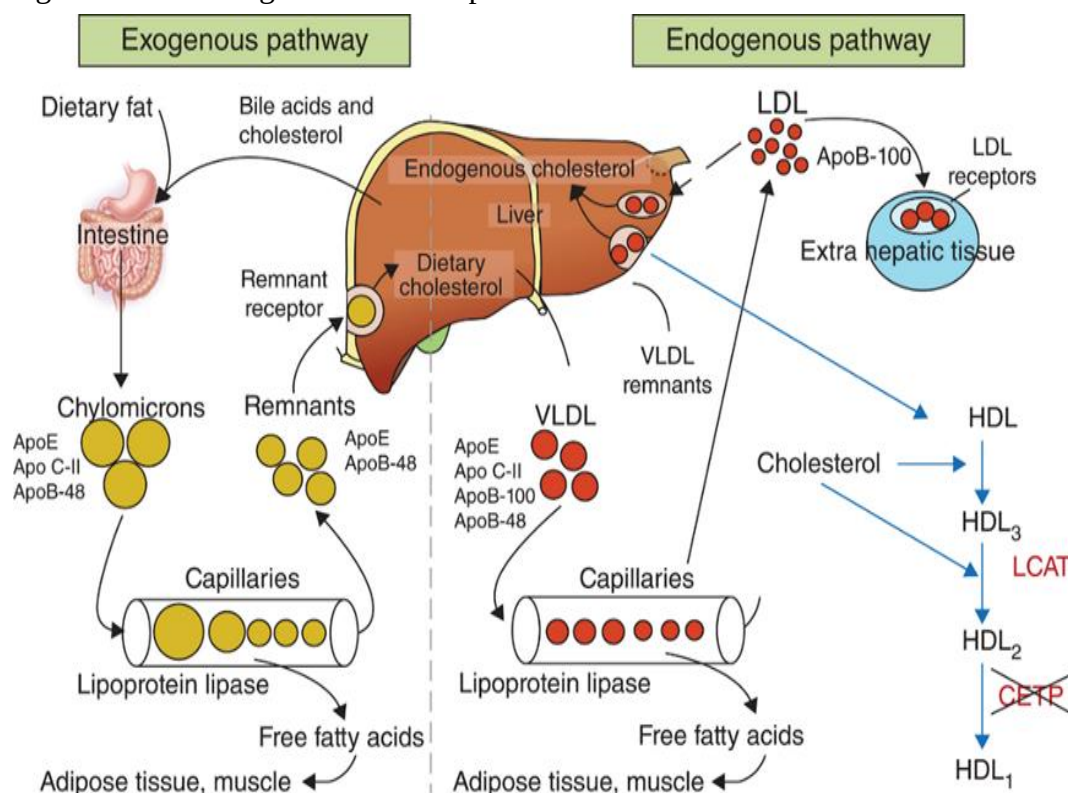


Fig: 1 - The diagram illustrates lipid metabolism via the exogenous and endogenous pathways. Dietary fats are absorbed and transported as chylomicrons, while the liver produces VLDL, which is converted to LDL for cholesterol delivery to tissues. HDL facilitates reverse cholesterol transport, regulated by LCAT and CETP enzymes.

Exogenous Pathway

The exogenous pathway starts with the intestinal absorption of triglycerides and cholesterol from dietary sources. Its end result is the transfer of triglycerides to adipose and muscle tissue and of cholesterol to the liver. After absorption, triglycerides and cholesterol are re-esterified in the intestinal mucosal cells and then coupled with various apoproteins, phospholipids, and unesterified cholesterol into lipoprotein particles called chylomicrons (11). The chylomicrons in turn are secreted into intestinal lymph, enter the

bloodstream through the thoracic duct, and bind to the wall of capillaries in adipose and skeletal muscle tissue. At these binding sites the chylomicrons interact with the enzyme lipoprotein lipase, which causes hydrolysis of the triglyceride core and liberation of free fatty acids. These fatty acids then pass through the capillary endothelial cells and reach the adipocytes and skeletal muscle cells for storage or oxidation, respectively (12). After removal of the triglyceride core, remnant chylomicron particles are formed. These are high in cholesterol esters and characterized by the presence of apoproteins B, CIII, and E. These

remnants are cleared from the circulation by binding of their E apoprotein to a receptor present only on the surface of hepatic cells. Subsequently, the bound remnants are taken to the inside of hepatic cells by endocytosis and then catabolized by lysosomes. This process liberates cholesterol, which is then either converted into bile acids, excreted in bile, or incorporated into lipoproteins originated in the liver (VLDL) (13). Under normal physiologic conditions, chylomicrons are present in plasma for 1 to 5 hours after a meal and may give it a milky appearance. They are usually cleared from the circulation after a 12-hour fast (14).

Endogenous Pathway

The liver constantly synthesizes triglycerides by utilizing as substrates free fatty acids and carbohydrates, these endogenous triglycerides are secreted into the circulation in the core of very-low-density lipoprotein particles (VLDL) (15). The synthesis and secretion of VLDL at cellular level occur in a process similar to that of chylomicrons, except that a different B apoprotein (B-100 instead of B-48) together with apoproteins C and E intervene in their secretion. Subsequent interaction of the VLDL particles with lipoprotein lipase in tissue capillaries leads to hydrolysis of the core triglycerides and production of smaller remnant VLDL particles rich in cholesterol esters (intermediate-density lipoproteins, IDL) and liberation of free fatty acids. Around half of these remnant particles are removed from the circulation in 2 to 6 hours as they bind tightly to hepatic cells (16). The rest undergo modifications with detachment of the remaining triglycerides and its substitution by cholesterol esters and removal of all the apoproteins except apoprotein B. This process results in transformation of the remnant VLDL particles into low-density lipoprotein particles (LDL) rich in cholesterol. In fact, these

last particles contain around three-fourths of the total cholesterol in human plasma, although they constitute only some 7% of the total cholesterol pool. Their predominant function is to supply cholesterol to cells with LDL receptors, like those in the adrenal glands, skeletal muscle, lymphocytes, gonads, and kidneys (17). The quantity of cholesterol freed from LDL is said to control cholesterol metabolism in the cell through the following mechanisms: (1) increased LDL cholesterol in the cell decreases synthesis of the enzyme 3-hydroxy-3 methyl glutaryl coenzyme A (HMG-CoA) reductase, which modulates the intracellular synthesis of cholesterol; (2) increased LDL cholesterol may enhance the storage of cholesterol within the cell by activation of another enzyme; and (3) increased cholesterol within the cell diminishes the synthesis of LDL receptors through a negative feedback process (18). Besides the above-described route for LDL degradation in extra hepatic sites, a so-called scavenger cell pathway has been described. This consists of cells in the reticuloendothelial system which, by phagocytosis, disposes of the excess concentrations of this lipoprotein in plasma (19).

Transport of High-Density Lipoprotein Cholesterol

High-density lipoproteins are a heterogeneous group of macromolecules with different physical properties and chemical components; two subclasses of HDL have been identified (HDL: and HDL3) within which several subspecies have also been demonstrated. The predomination function of HDL seems to be the reverse transport of cholesterol from different tissues into the liver, where it is eventually removed. Subclass HDL has been reported to have a better correlation with coronary artery disease protection than total HDL cholesterol (20). The serum concentration of HDL and its components derives from various complex



intravascular and cellular metabolic events. These events include secretion of precursor HDL particles from the liver and small intestine, interaction of these particles with lipids and proteins released during the catabolism of triglyceride-rich lipoproteins, and production of cholesterol esters (the core substance in HDL) from the action of lecithin-cholesterol acyltransferase (LCAT), an enzyme that originates in the liver (21). This enzyme acts on unesterified cholesterol released into plasma from cellular turnover. The cholesterol esters formed in this reaction are in turn transferred to VLDL and subsequently appear in LDL. The end result is a system that allows the transfer of cholesterol through LDL to peripheral cells and its return to the liver through HDL, and that prevents excessive accumulation of cholesterol in the body (22).

Pathophysiology of Hyperlipidaemia

Hypercholesterolemia develops as a consequence of abnormal lipoprotein metabolism, mainly reduction of LDL receptor expression or activity, and consequently diminishing hepatic LDL clearance from the plasma. It is a major predisposing risk factor for the development of atherosclerosis (23). This mechanism is classically seen in familial hypercholesterolemia and when excess saturated fat or cholesterol is ingested. In addition, excessive production of VLDL by the liver, as seen in familial combined hyperlipidaemia and insulin resistance states such as abdominal obesity and Type II diabetes, can also induce hypercholesterolemia or mixed dyslipidaemia (24).

A current theory for the initiating event in atherogenesis is that apoprotein B-100 containing lipoproteins are retained in the sub endothelial space, by means of a charge-mediated interaction with extracellular matrix and proteoglycans. This allows reactive oxygen species to modify the

surface phospholipids and unesterified cholesterol of the small LDL particles (25). Circulating LDL can also be taken up into macrophages through unregulated scavenger receptors. As a result of LDL oxidation, isoprostanes are formed. Isoprostanes are chemically stable: free radical catalysed products of arachidonic acid, and is structural isomers of conventional prostaglandins. Isoprostanes levels are increased in atherosclerotic lesions, but they may also be found as F2 isoprostanes in the urine of asymptomatic patients with hypercholesterolemia (26). A strong association exists between elevated plasma concentrations of oxidized LDL, and CHD. The mechanisms through which oxidized LDL promotes atherosclerosis are multiple and include damage to the endothelium, induction of growth factors, and recruitment of macrophages and monocytes (27). Vasoconstriction in the setting of high levels of oxidized LDL seem to be related to a reduced release of the vasodilator nitric oxide from the damaged endothelial wall as well as increased platelet aggregation and thromboxane release. Smooth muscle proliferation has been linked to the release of cytokines from activated platelets (28). The state of hypercholesterolemia leads invariably to an excess accumulation of oxidized LDL within the macrophages, thereby transforming them into "foam" cells. The rupture of these cells can lead to further damage of the vessel wall due to the release of oxygen free radicals, oxidized LDL, and intracellular enzymes (29). This is a metabolically complex disease of lipid-lipoprotein metabolism and the exact etiology is not fully appreciated. The familial type in schnauzers may involve defects lipoprotein lipase and/or Apoprotein C-11, a required cofactor for lipoprotein lipase activity. This defect causes a failure to breakdown chylomicrons and VLDL, and results in excessive levels of circulating triglycerides. It is the elevated concentration of



triglycerides that is responsible for the clinical signs (30).

Sign and Symptoms of Hyperlipidaemia

Symptoms of hyperlipidaemia are not noticeable and discovered during daily examination or evaluation (31, 32).

- Xanthoma
- Xanthelasma of eyelid
- Chest Pain
- Abdominal Pain
- Enlarged Spleen
- Liver Enlarged
- High cholesterol or triglyceride levels
- Heart attack
- Higher rate of obesity and glucose intolerance
- Pimple like lesions on body
- Atheromatous plaques in the arteries
- Arcussenilis
- Xanthomata

Classification of Hyperlipidemia:

Primary Hyperlipidemia: This occurs as a result of high intake of diet, rich in saturated fats and cholesterol or because of some genetic defect and heredity factor.

Secondary Hyperlipidemia: This occurs as a result of some other illnesses or metabolic disturbances, e.g., diabetes mellitus, hypothyroidism, obstructive liver disease. When hyperlipidemia is defined in terms of a class or classes of elevated lipoprotein in the blood, the term "Hyperlipoproteinemia" is used. "Hypercholesterolemia" is the term for high cholesterol levels in the blood (desirable: <200 mg/dl; borderline: 200 to 239 mg/dl; high: > 240 mg/dl). "Hypertriglyceridemia" refers to high

triglyceride levels in the blood (desirable: <200 mg/dl; borderline: 200 to 400 mg/dl; high 400 to 1000 mg/dl; very high: >1000 mg/dl), while the "Mixed Hyperlipidemia" is used to indicate increased cholesterol and triglyceride levels.

Consequences of Hyperlipidemia:

Atheroma:

Atherosclerosis is the major cause of cardiovascular disease. Atherosclerosis may be defined as degenerative changes in the intima of medium and large arteries. It is characterized by a focal deposit of cholesterol and lipids, primarily within the internal wall of the artery and is followed by the formation of fibrous tissues and calcium deposition in the intima of blood vessels (Monireh et al. 2014) (33). The genesis of plaque formation is the result of complex interactions between the components of the blood and the elements forming the vascular wall. Atherosclerotic plaque forms through the distribution of cholesterol from the liver to the tissues and this is carried out by LDL cholesterol. A relationship is established between the elevated plasma lipids and the development of atherosclerotic plaques. When inner layer of artery thicken with accumulation of cells, connective tissues, lipids and debris, it is called as atheroma. It is preceded by accumulating or absorbing foam cells i.e. macrophages loaded with lipids. A fatty streak form due to activation of nuclear factor kappa B pathway is the initiating of inflammatory response. These inflammatory responses cause release of interleukin-1 β and TNFa factors. They promote cell adhesion of molecules by bridging between lipoprotein particle and matrix proteoglycans, which results in accumulation of lipoprotein in vessels (34).

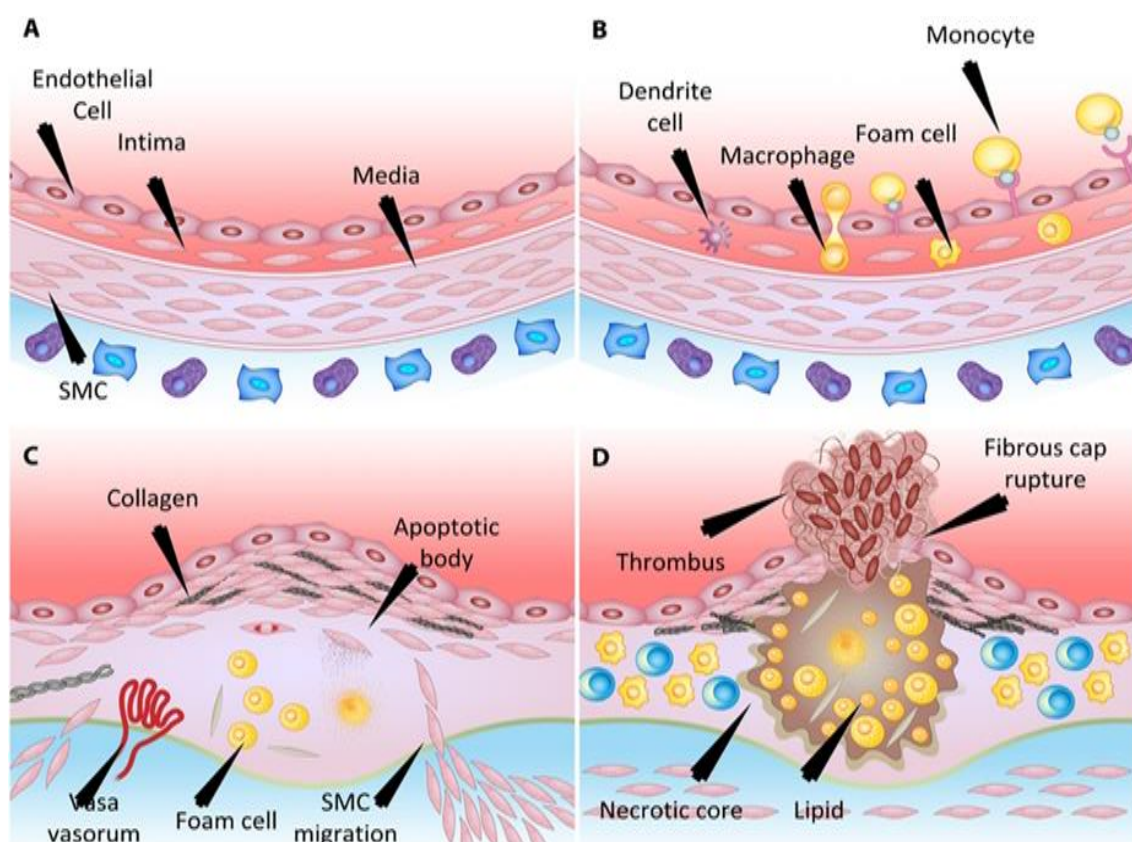


Fig 2 :- The figure depicts the progression of atherosclerosis. (A) Normal arterial wall structure. (B) Early lesion formation with immune cell infiltration and foam cell development. (C) Advanced plaque with smooth muscle cell (SMC) migration and extracellular matrix deposition. (D) Plaque rupture leading to thrombus formation and potential vascular occlusion.

Myocardial infarction:

In normal body condition, circulating estrogen causes production of nitric oxide synthase which inhibits Ca^{2+} channels and activate mitogen activated protein kinases (MAPK) and protect heart from myocardial infarction. Obesity cause abnormal level of circulating estrogen and adiponectin. It also cause increase in plasminogen activator inhibitor activity (35). In atheroma, artery thickens by preventing blood flow and forms plaque by accumulating tissues, lipid and debris. Further, this plaque occludes to formation of thrombus (36).

High blood pressure:

Hyperlipidemia increases amount of FFA in our body and its level become abnormal. So, the lipids get accumulated in to peripheral tissues and activate the adrenergic receptors. This increases vascular adrenergic sensitivity and vascular tone. Also, inhibition of $\text{Na}^{+} \text{K}^{+}$ ATPase and sodium pump occurs and there is an increase in heart rate and further high blood pressure occurs (37).

Diabetes:

Diabetes is a metabolic disorder. Normally, liver produce triglycerides from FFA and is required for gluconeogenesis. By the process of lipolysis, FFA is released from triglycerides and leads to the oxidation and release energy in muscle cells or converted them into lipoproteins (38). Insulin is the key hormone which regulates this process. In

case of hyperlipidemia, amount of FFA is increased and cause the insensitivity of adipocytes to insulin. This leads to diabetes mellitus and glucose intolerance. Also in obesity, there are chemokines secretions promoting macrophage infiltration and activation. This produces cytokines and impact on insulin sensitivity. Moreover, drugs which are given for diabetes mellitus, lower the glucose levels and lead to weight gain. Obesity causes change in or alteration in hormones secreted from adipose tissues which causes increase in fatty acid metabolites accumulation in body, so there is development of insulin resistance and sometimes it may cause beta cell dysfunctioning (39).

Osteoarthritis:

Mechanical changes in knee and articular cartilage are occurred because of Obesity. Knee joint cannot balance overweight of body (40). Small change or increase in body weight causes increase in risk factor of osteoarthritis. In this leptin levels increased which causes activation of degradative enzyme. Further obesity causes activation of mechanoreceptors within cartilage. So, the production of growth factors, cytokines, nitric oxide, prostaglandins etc. increased which leads to degeneration of neurons. Somewhat it also affects on spinal mechanism, so, compressive force and anterior force on spinal column (41).

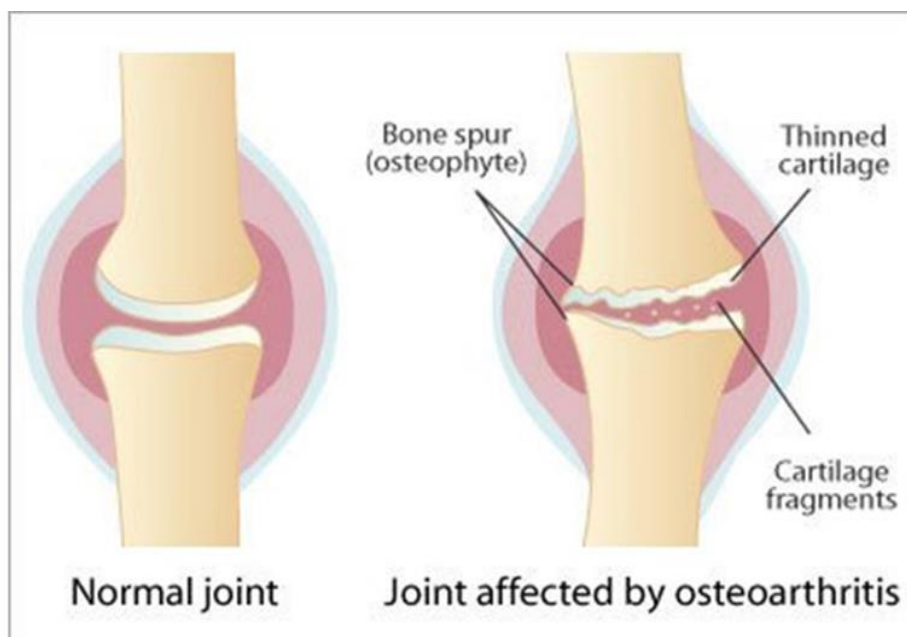


Fig 3:- The illustration compares a normal joint with one affected by osteoarthritis. The osteoarthritic joint shows thinned cartilage, cartilage fragments, and the formation of bone spurs (osteophytes), leading to joint degeneration and inflammation.

Hyperlipidemia Treatment

Current Drug Targets Against Hyperlipidemia

Activators of Peroxisome Proliferator-Activated Acceptor

The peroxisome proliferator-activated receptors (PPARs) are members of the nuclear receptor super family that function as fatty acid-activated transcription factors (42). PPARs are regulators of numerous metabolic pathways; hence there is huge increase in the development and use of agonists of these receptors as therapeutics for diabetes, dyslipidemia, and atherosclerosis (43).

Three different PPAR genes (α, β/δ, and γ) have been identified, each isoform displaying distinct patterns of tissues distribution and specific pharmacological activators, performing their distinct functions in different cell types (44). PPARα is mostly expressed in the tissues involved in lipid oxidation, such as liver, kidney, skeletal, cardiac muscle, and adrenal glands, PPARα potentiates FAs oxidation in the liver, heart, kidney, and skeletal muscle. Activation of PPARα leads to an increase in expression of lipoprotein lipase and apoA-V and to a decrease in hepatic apoC-III. These actions lower plasma TGs in chylomicrons and VLDL particles, thus liberating FAs, which are taken up and stored as fat in adipocytes or metabolized in skeletal muscle. In addition, PPARα activation increases hepatic apoA-1 and -11 expression, which raises HDL cholesterol levels, and promotes HDL-mediated cholesterol efflux from macrophages by inducing ATP-binding cassette A1 transporter (45). PPARγ is expressed in adipose tissue, macrophages, and vascular smooth muscles, while PPARδ is mainly expressed in skeletal muscle and adipose tissues. PPARβ/δ is best known for its role in skin homeostasis, and has recently been shown to play a role in HDL metabolism. A combination of PPARα and PPARγ agonists would be expected to achieve beneficial effects on restoring metabolic disorders. Hence, a number of PPARα/γ dual agonists have been designed and developed. However, recently identified PPARα/γ dual agonists were ineffective because of undesirable side effects during preclinical or clinical trials. For example, muraglitazar, a synthetic PPARα/γ dual agonist, was aborted during clinical trials because of increased mortality, fluid retention, edema, and cancer (46). PPARα regulates genes involved in FA uptake, β-oxidation, and ω-oxidation and down-regulates apolipoprotein C-III, a protein that inhibits TG hydrolysis by lipoprotein lipase, and it also regulates genes involved in reverse

cholesterol transport, such as apolipoprotein A-I and A-II. PPARα and PPARγ are the molecular targets of number of marketed drugs such as fibrates, the activator of PPARα and the thiazolidinediones, the activators of PPAR γ (47).

Cholesterol Ester Transfer Protein Inhibitors

Cholesterol ester transfer protein (CETP) is a plasma glycoprotein that facilitates the movement of cholesterol esters and triglycerides between the various lipoproteins in the blood by mediating the transfer of cholesterol esters from the cardio protective HDL-c to the proatherogenic LDL-c and VLDL-c (48). Thus, the movement of cholesterol esters from HDL-to LDL-c by CETP has the overall undesirable effect of lowering HDL-c. It therefore follows that inhibition of CETP should lead to elevation of plasma HDL-c and lowering of plasma LDL-C, thereby providing a therapeutically beneficial plasma lipid profile (49). Elevation in HDL levels is equally favored by diminished CETP-mediated transfer of CE and HDL to atherogenic acceptor lipoproteins (i.e. VLDL, LDL). Elevated CETP activity is a major player whose action underlies the atherogenic particle profile of both LDL and HDL in Type II diabetes (50). Inhibition of CETP, a key protein involved in reverse cholesterol transport, can consequently lead to increases in HDL-c levels and thus, is under evaluation as an anti-atherogenic strategy. To date, anacetrapib demonstrates the greatest HDL-c raising and LDL c lowering potential (51). There are three CETP inhibitors that have been used in clinical trials. Tercetrapib was the first to go into human trials but was discontinued in Phase III because of excessive rates of mortality in the ILLUMINATE (investigation of lipid level management to understand its impact in atherosclerotic events) trial. Anacetrapib, which has a similar structure to torcetrapib but does not share its properties when

it comes to the effects on aldosterone production, is presently in Phase III research. Dalcetrapib, which is structurally different than tercetrapib, is currently undergoing cardiovascular outcomes trials (52), 2-Arylbenzoxazole, (53), tetrahydroquinoline (BAY 38-1335) (54), chromanol derivatives, and 2-(4-carbomylphenyl) benzoxazole are under development as CEPT inhibitors (55-57).

Cholesterol absorption inhibitors

Ezetimibe is the only drug currently available from this class whose mechanism of action involves inhibition of dietary cholesterol absorption without affecting the absorption of fat-soluble vitamins, triglycerides, and bile acids. Ezetimibe binds to cholesterol transporter NPLILI (Niemann-pick C1-like) protein in the brush border of intestine as well as in hepatocytes (58). Decrease in cholesterol absorption leads to compensatory up-regulation of LDL receptors on the cell surface and increased LDL cholesterol uptake into cells and decreases blood LDL cholesterol content. Ezetimibe also exerts anti-inflammatory effect and also appears to improve renal function (59). Some side effects of ezetimibe are diarrhoea, abdominal pain, arthralgia, backache, myalgia, headache, sinusitis, hepatitis, anaphylaxis, myopathy, and rhabdomyolysis. This drug is contraindicated in active liver diseases. Ezetimibe is primarily metabolized in the small intestine and liver via glucuronide conjugation with subsequent biliary and renal excretion. oral administration, ezetimibe is absorbed and extensively conjugated to a pharmacologically active phenolic glucuronide (ezetimibe glucuronide), the drug and its metabolite have a half-life of approximately 22 hours (60).

Squalene synthase inhibitors

Squalene synthase, a key enzyme in the cholesterol biosynthetic pathway, occupies the first and solely committed step towards the biosynthesis of the sterol nucleus of cholesterol hence it is an attractive target for inhibition and the development of novel and improved antihypercholesterolemic agents (61). Squalene synthase catalyzes one of the subsequent reactions in the cholesterol biosynthetic pathway (i.e. it reductively dimerizes two farnesyl pyrophosphate molecules to form squalene) which is the first intermediate committed to cholesterol (62). Squalene synthase inhibitors are emerging new stars in the hypolipidemic drug sky and represent a novel class of antihyperlipidemics. Squalene synthase is implicated in the late step in cholesterol biosynthesis and, the squalene synthase inhibitors exerts same effect as that of 3-hydroxy-3-methylglutaryl-coenzyme A-CoA reductase inhibitors, with decreased cholesterol production and upregulation of LDL receptors (63). Early inhibitors such as the zaragozic acids showed significant toxicity (acidosis), but a recent compound, lapaquistat, reached Phase III clinical trials (64). EP2306 and EP2302 have been shown to possess antioxidant properties both in vitro and in vivo (65) as well as to inhibit squalene synthase activity and lipid biosynthesis in vitro (66).

Cholesterol Metabolizing Cytochrome P450

Implication for cholesterol lowering from the family of P450s, the 7A1, 27A1 and 46A1 are the most important enzymes involved in the control of cholesterol levels in the periphery and brain (67), CYP7A1 is an important determinant of plasma cholesterol levels and is considered as target for cholesterol lowering (68). CYP27A1 converts cholesterol to 27-hydroxycholesterol by oxygenation reaction and this is suggested to be important reaction for cholesterol elimination

from human lung macrophages and cells in arterial endothelium (69).

AMP-Activated Protein Kinase Activator

AMP-activated protein kinase (AMPK), a heterotrimeric energy sensing protein, which restores cellular energy balance by promoting ATP-generating pathways (e.g. FA oxidation) and inhibiting ATP-utilizing pathways (e.g. FA synthesis) (70). AMPK system plays a major role in regulating glucose and lipid metabolism by effect on energy metabolism and long-term effect on gene expression in the liver (71). In liver,

activation of AMPK results in decreased production of plasma TG and cholesterol and enhanced FA oxidation (72). WS070117 is synthetic lipid lowering agent that is approved preclinical as an effective activator of AMPK with potential capability of inhibition of de novo hepatic lipogenesis (73).

Pharmacological therapy

The mechanism of action and profile of lipid lowering effect of important antihyperlipidemic drugs is summarized in Table (74).

Table: Classification of synthetic drugs

Sr. No.	Class of drug	Name of the drugs	Mechanism of action	Effects on lipoproteins
1	HMG-CoA reductase inhibitors	Lovastatin Simvastatin Pravastatin Atorvastatin Rosuvastatin	Inhibit rate limiting enzyme and decrease synthesis of cholesterol	LDL↓ HDL↑ TG ↓
2	Bile acid sequestrants	Cholestyramine Colestipol	Binds to the bile acids in intestine, increased hepatic metabolism of cholesterol	LDL↓ HDL↑ TG may be increased or decreased
3	Activate lipoprotein lipase	Clofibrate Gemfibrozil Bezafibrate Fenofibrate	The activity of lipoprotein lipase enzyme increased and reduction in the release of fatty acids from adipose tissues.	LDL↓ HDL↑ TG ↓
4	Inhibition of lipolysis and triglyceride synthesis	Nicotinic acid	Decrease in lipolysis of adipocytes and production of VLDL	LDL↓ HDL↑ TG ↓

Herbal Medicines

Herbal medicines are the synthesis of therapeutic experiences of generations of practicing physicians of indigenous systems of medicine for over hundreds of years while nutraceuticals are nutritionally or medicinally enhanced foods with health benefits of recent origin and marketed in

developed countries (75). The marketing of the former under the category of the latter is unethical. Herbal medicines are also in great demand in the developed world for primary health care because of their efficacy, safety and lesser side effects (76).

Nigella sativa



Medicinal plants have been used for curing diseases for many centuries in different indigenous systems of medicine as well as folk medicines. Moreover, medicinal plants are also used in the preparation of herbal medicines as they are considered to be safe as compared to modern allopathic medicines. Many researchers are focusing on medicinal plants since only a few plant species have been thoroughly investigated for their medicinal properties, potential, mechanism of action, safety evaluation and toxicological studies. Among various medicinal plants, *Nigella sativa* (*N. sativa*) (Family Ranunculaceae) is emerging as a miracle herb with a rich historical and religious background since many researches revealed its wide spectrum of pharmacological potential. *N. sativa* is commonly known as black seed. *N. sativa* is native to Southern Europe, North Africa and Southwest Asia and it is cultivated in many countries in the world like Middle Eastern Mediterranean region, South Europe, India, Pakistan, Syria, Turkey, Saudi Arabia (77). *N. sativa* has been extensively studied for its biological activities and therapeutic potential and shown to possess wide spectrum of activities viz. as hyperlipidemia, diuretic, antihypertensive, antidiabetic, anticancer and immunomodulatory, analgesic, antimicrobial, anthelmintics, analgesics and anti-inflammatory, spasmolytic, bronchodilator, gastroprotective, hepatoprotective, renal protective and antioxidant properties. The seeds of *N. sativa* are widely used in the treatment of various diseases like bronchitis, asthma, diarrhoea, rheumatism and skin disorders. It is also used as liver tonic, digestive, anti-diarrheal, appetite stimulant, emmenagogue, to increase milk production in nursing mothers to fight parasitic infections, and to support immune system (78,79). Dyslipidemia is an established risk factor for ischemic heart disease. *Nigella sativa* (NS) is a medicinal plant that has been used

for the treatment and prevention of a variety of diseases, in particular hyperlipidemia.

Classification

Scientific classification *Nigella sativa*

Scientific classification	
Kingdom	Plantae
Clade	Angiosperms
Clade	Eudicots
Clade	Ranunculales
Family	Ranunculaceae
Genus	<i>Nigella</i>
Species	<i>Nigella sativa</i> L.



Figure: 4 - *Nigella Sativa*

❖ Aim and objective:

❖ Aim

To explore the therapeutic potential of *Nigella sativa* phytoconstituents in the treatment of hyperlipidemia using an integrative approach of network pharmacology, molecular docking, and ADMET analysis.

Objectives

1.To identify and retrieve the active phytochemicals present in *Nigella sativa* using phytochemical databases such as IMPPAT and PubChem.

2.To evaluate the drug-likeness and pharmacokinetic properties (ADME) of these

phytoconstituents using Lipinski's Rule of Five and related filters.

3. To predict and map the potential molecular targets of *Nigella sativa* phytoconstituents using Swiss Target Prediction and correlate them with hyperlipidemia-related genes using databases like Gene Cards and DisGeNET.

4. To analyze the common gene targets using Venny and construct a protein-protein interaction (PPI) network via STRING and Cytoscape.

5. To perform pathway enrichment analysis (GO and KEGG) for understanding the molecular mechanisms involved in lipid metabolism and hyperlipidemia.

6. To conduct molecular docking studies to determine binding affinities between selected phytoconstituents and key protein targets like PPARA and PYGL.

7. To evaluate the safety and bioavailability profiles (ADMET) of the top compounds for drug candidacy.

8. To validate and interpret findings for identifying lead compounds with strong anti-hyperlipidemic potential suitable for further experimental development.

Plan of Work



MATERIAL AND METHOD

5.1. Network Pharmacology:

1. Botanical Identification and Recovery of Active Ingredients:

The phytoconstituents of the various botanicals were identified through literature review and

IMPPAT (Indian Medicinal Plants, Phytochemistry and Therapeutics) (mochimic.res.in/imppat), as well as Dr. Duke's Phytochemical and Ethnobotanical Database (<http://phytochem.nal.usda.gov/>). The botanicals were identified through previous literature. Next, the Structure data file (SDF) format of the active



chemicals was retrieved from PubChem database (Chakkittukandivil et al., 2023)

2.Examination of Phytochemicals for Potential Drug-Like Qualities:

The collected phytochemicals were assessed using Lipinski's rule of five and the ADMET Characteristics (absorption, distribution, metabolism, excretion, and toxicity). Software like Prop was used to help with the ADMET study. For additional research, phytoconstituents exhibiting the designated range for several parameters, such as molecular weight, water partition coefficient, blood partition coefficient, etc., were chosen (Khanna et al., 2024).

3.Phytotarget and Disease Target Identification:

Use databases such as Swiss Target Prediction (<https://www.swisstargetprediction.ch/>), which predicts targets based on structural characteristics of compounds and experimental data on the compounds binding properties to different proteins, the therapeutic targets for each active phytoconstituents were found. These databases used the canonical SMILES of the phytoconstituents as input to obtain the appropriate targets. DisGeNET database (<https://www.disgenet.org/>) helped identify the treatment targets of disease and provides a comprehensive and evidence-based view of the relationship between diseases and genes (Khanna et al., 2024).

4.Common Targets Identification:

The online Venny 2.1.0 tool was used to identify common targets by analyzing the relationships between different datasets. By adding the therapeutic targets of disease after the targets of the chosen phytochemicals, the common targets

were found. The shared targets are the output of the Venny tool's analysis of the two sets of data (Zhang et al., 2021).

5.Target Network Construction and Topological Analysis:

A PPI network was established by selecting "Homo sapiens" as the species and adding the common targets found using the Venny 2.1.0 tool to the STRING database. This comprehensive database provides a comprehensive overview of PPIs and functional links, facilitating an understanding of the interactions between various proteins. Then, with the aid of the topological analysis, different biological networks could be visualized and analyzed using Cytoscape software. Degree Centrality (DC) and Betweenness Centrality (BC), two topological parameters, were evaluated using the CytoNCA plugin for Cyscape, On the basis of these topological criteria, the top proteins were identified (Zhang et al., 2021).

6. Gene Ontology (GO) Enrichment Analysis:

The FunRich software, a helpful tool for visualizing and analyzing functional enrichment in genes and proteins, was used to assist with the Gene Ontology (GO) enrichment study. It's a useful tool for analyzing the proteome and genetic data's functional significance. For molecular function (MF), cellular process (CP), and biological process (BP), an enrichment analysis was performed (Liu et al., 2023).

7.Pathway Enrichment Analysis:

The Reactome software, a comprehensive database of biological pathways and activities, was used to assist with the pathway enrichment study. It is widely utilized in many scientific contexts, such as drug development, disease research, and



systems biology for pathway analysis and visualization (Liu et al., 2023).

5.2. Molecular Docking:

Three-dimensional structures are used in molecular docking, an inexpensive, safe, and user-friendly technique that facilitates the interpretation, identification, and study of molecular properties. The structural interactions between two or more molecules of a chemical are predicted using a method known as docking. The method is applicable to computational chemistry, computer-aided biology, and molecular systems ranging in size from tiny molecules to large biomolecules and material assemblies. Currently, the primary focus of docking research is the interaction between a flexible ligand and a physiological receptor (Raval and Ganatra, 2022).

1) Target Protein Determination and Preparation:

The process of docking begins with obtaining the protein's 3D structure, preferably bound by a ligand, from the PDB. High-resolution or high-affinity ligand structures are recommended; however, this may not apply to all proteins. Molecular docking requires the specification of certain parameters. Hydrogens must be added, water must be removed, charges must be assigned and energy minimization must be done during protein preparation. Several reparation modules are available to address common issues with PDB files. The software-specific parameterization techniques used differ. AutoDock and SwissDock employ an program force field (Muhammed and Aki-Yalcin, 2024).

2.Preparation of Ligand:

The ligand structures obtained from chemical libraries or databases such as PubChem Energy

minimization has to be done before using these structures in docking. It is advised to visually inspect the target and ligand preparations' outcomes. Because erroneous connections, missing bonds, and aberrant geometries can result from certain preparation techniques in molecular descriptions. These mistakes frequently happen while converting molecules between different formats. It therefore spreads quickly. Once the ligand and target are ready, the binding site needs to be identified and restricted. This phase can be completed using the coordinates of a ligand that is coupled to the protein, or by manually specifying the coordinates. The docking calculations' center, the binding area, is mapped using the grid. The probe atoms in the grid represent the outline of a potential interaction, and the grid itself can be compared to a box with known dimensions that has been divided into tiny squares. Docking outcomes are impacted by grid size and resolution (Trott and Olson, 2010).

3. Selection of the Best Docking Scoring Function:

The stability of the ligand-protein combination determines whether docking scoring function is optimal. Selecting an appropriate scoring function that provides an accurate binding pattern and a potential ligand is challenging. According to theory, a protein-ligand combination is more stable the lower its binding free energy (ΔG). Numerous programs calculate the docking score in order to quickly detect and rank multiple ligand poses. Binders and nonbinders should be easily distinguished using scoring functions. Furthermore, it must be able to quickly and accurately distinguish between the proper and improper binding modalities of a ligand. There are three primary classifications for scoring functions: knowledge-based, force field, and empirical (Muhammed and Aki-Yalcin, 2024)



4. Docking Validation:

Docking process validation is just as important as any other technique. Verifying the docking results involves redocking reference ligands with targets and contrasting the estimated bindings' coverage, RMSD (root mean square deviation), binding posture, and binding affinity with previously obtained data. Molecular dynamics studies should be performed if the structures of the ligand and the target are complicated. Calculating the binding free energy including the solvent effect, fixing the complex after docking, providing flexibility, and ensuring the precise sequence of putative ligands can all be accomplished via molecular dynamics simulations. The ligands' binding positions, binding residues, and binding energies are disclosed at the conclusion of the process (Counia et al., 2017).

RESULT:

6.1 Network Pharmacology

1) Active components in *Nigella Sativa*:

A literature review, along with data from the IMPPAT (Indian Medicinal Plants, Phytochemistry and Therapeutics) database,

revealed that *Nigella Sativa* contains 192 active components. The selected components were further subjected to an evaluation of the pharmacokinetic parameters. The ADME criteria for optimum bioavailability is defined as having a value of 30% or greater, while the drug-likeness must exceed 0.18. These thresholds are essential for evaluating the pharmacokinetic properties and potential efficacy of a drug candidate.

2) Screening of Components of *Nigella Sativa* for Hyperlipidemia:

Among the 192 components, only 5 components were found to relate with hyperlipidemia. The genes of each active component were fetched from Swiss target prediction genes of the disease ie, hyperlipidemia were retrieved from Gene Cards man database specific to Homo sapiens were selected. Using a Venn diagram as shown in (Figure 5.1), 3 common targets were identified between the compound targets 89 and hyperlipidemia-related targets 44. These common targets were as shown in following (Table 5.1)

Table 5.1: Common Gene of Hyperlipidemia

Gene Symbols of Common gene (3)
PPARA, PYGL, CYP19A1

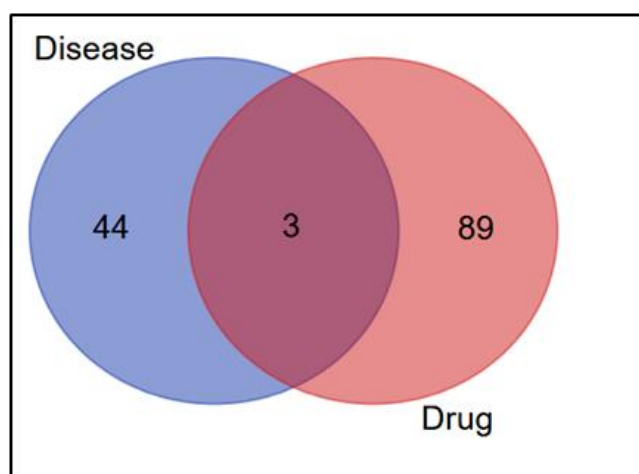


Figure 5.1: Venn diagram of common gene from Target Gene and Disease Gene through Network Pharmacology

3) Construction and Analysis of Target PPI Network:

To enhance the visualization and interpretation of the underlying molecular mechanisms, an in-depth analysis of protein-protein interactions (PPI) was conducted for the identified target genes. These genes, associated with specific bioactive compounds, were analyzed using STRING v11, a widely recognized database and tool for constructing and visualizing PPI networks. The analysis provided an integrated perspective on the functional interrelationships among the target proteins and their involvement in biological pathways pertinent to hyperlipidemia. To ensure the reliability of the interactions, the network was filtered to retain only high-confidence interactions with a combined score > 0.9 , thereby emphasizing the most robust and biologically relevant associations. The resulting PPI network, illustrated in Figure 5.2, highlights the connectivity and

interaction dynamics among the identified targets. The local clustering coefficient of the network was calculated to be 0.69, indicating a relatively high degree of interconnectedness among the nodes, which reflects the potential for cooperative or co-regulated functional roles within the disease context. To identify the most central and functionally important proteins within the network, the CytoHubba plugin in Cytoscape v3.10.0 was employed. Hub genes were prioritized using topological analysis methods such as Density of Maximum Neighborhood Component (DMNC) and Maximum Neighborhood Component (MNC). This analysis revealed a set of core targets implicated in hyperlipidemia pathogenesis, including PYGL, PPARA, ACTN4, CHRN5, LEPER, NPHS1, NOS3, CFHR1 ABCY3, and CYP19A1. These hub proteins may serve as critical nodes in the regulatory network and represent potential therapeutic targets for intervention.

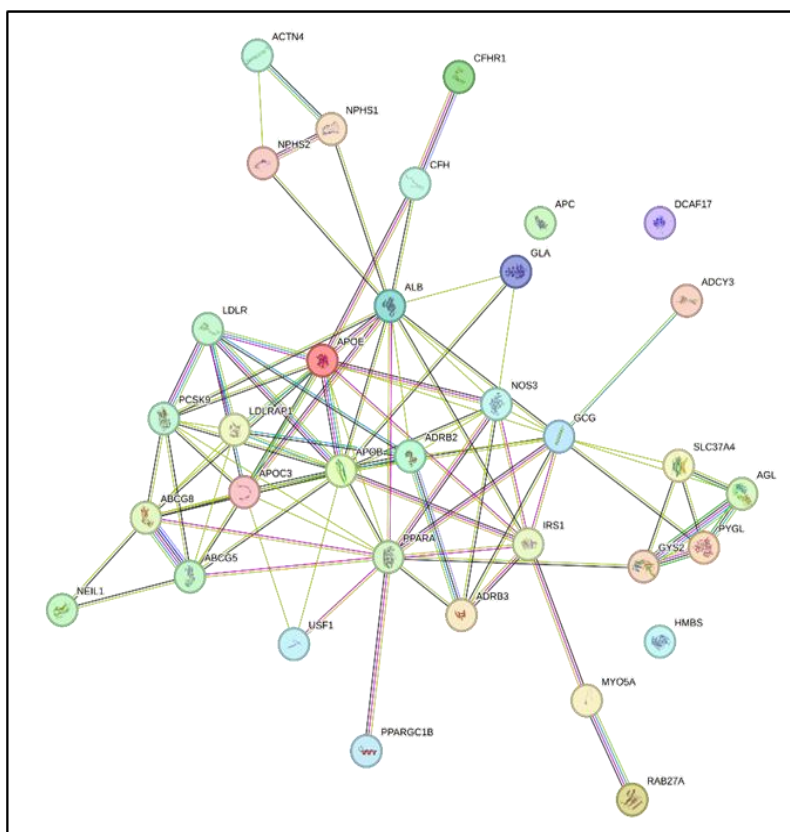


Figure 5.2: Protein-Protein interactions analyzed using STRING v11

Top Hub Gene:

Genes have many interaction with other genes that are hub genes, more interaction to other genes

(more degree) and less interaction to other gene are (less degree) considered. Top ten were selected for the Network Pharmacology as shown in (Figure 5.3).

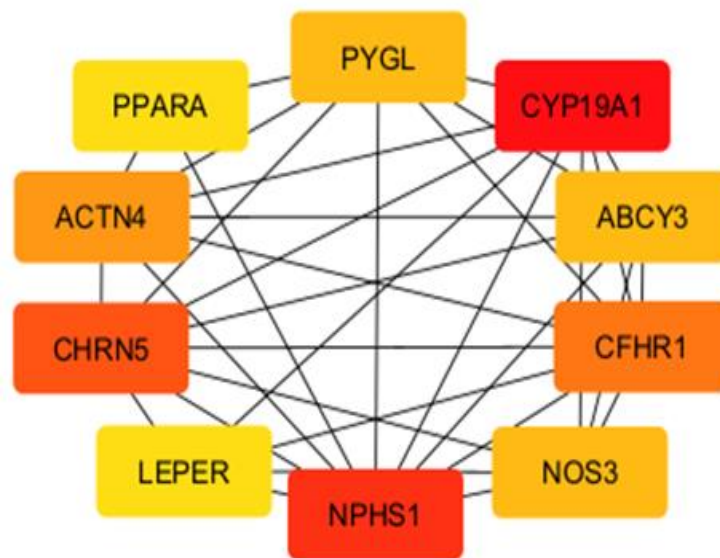


Figure 5.3: Top 10 Gene Obtained From Cytoscape Ranked By Degree Method.

5) Construction of Compound-Target Network:

A compound-target network was constructed using Cytoscape to explore the relationships between the identified compounds and their associated target genes as shown in (Figure: 5.4). This analysis allowed for a deeper understanding of the signaling pathways and potential therapeutic uses of the compounds identified in the extract. In compound-target-disease interaction network to explore how drugs work in treating

hyperlipidemia. Our analysis showed that several targets were impacted by multiple compounds, suggesting that these active biochemical substances might exert a synergistic effect on these targets. This observation also implies that the compounds could potentially offer therapeutic benefits for other diseases and disorders beyond just hyperlipidemia. The network analysis reveals several important proteins with high degrees that are critical targets in hyperlipidemia and some compounds may have significant pharmacological effects on other diseases.

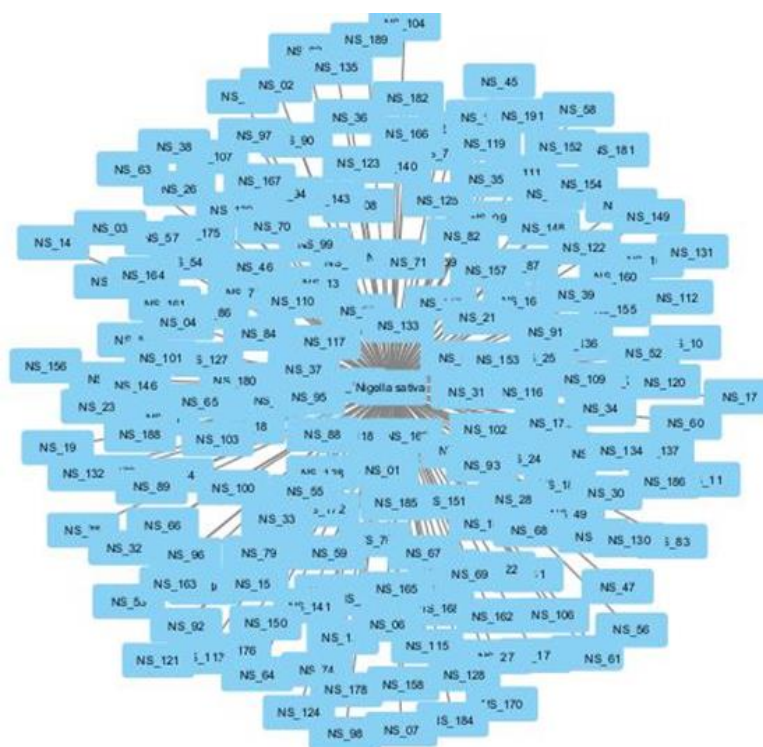


Figure 5.4: Cytoscape visualization target pathway network.

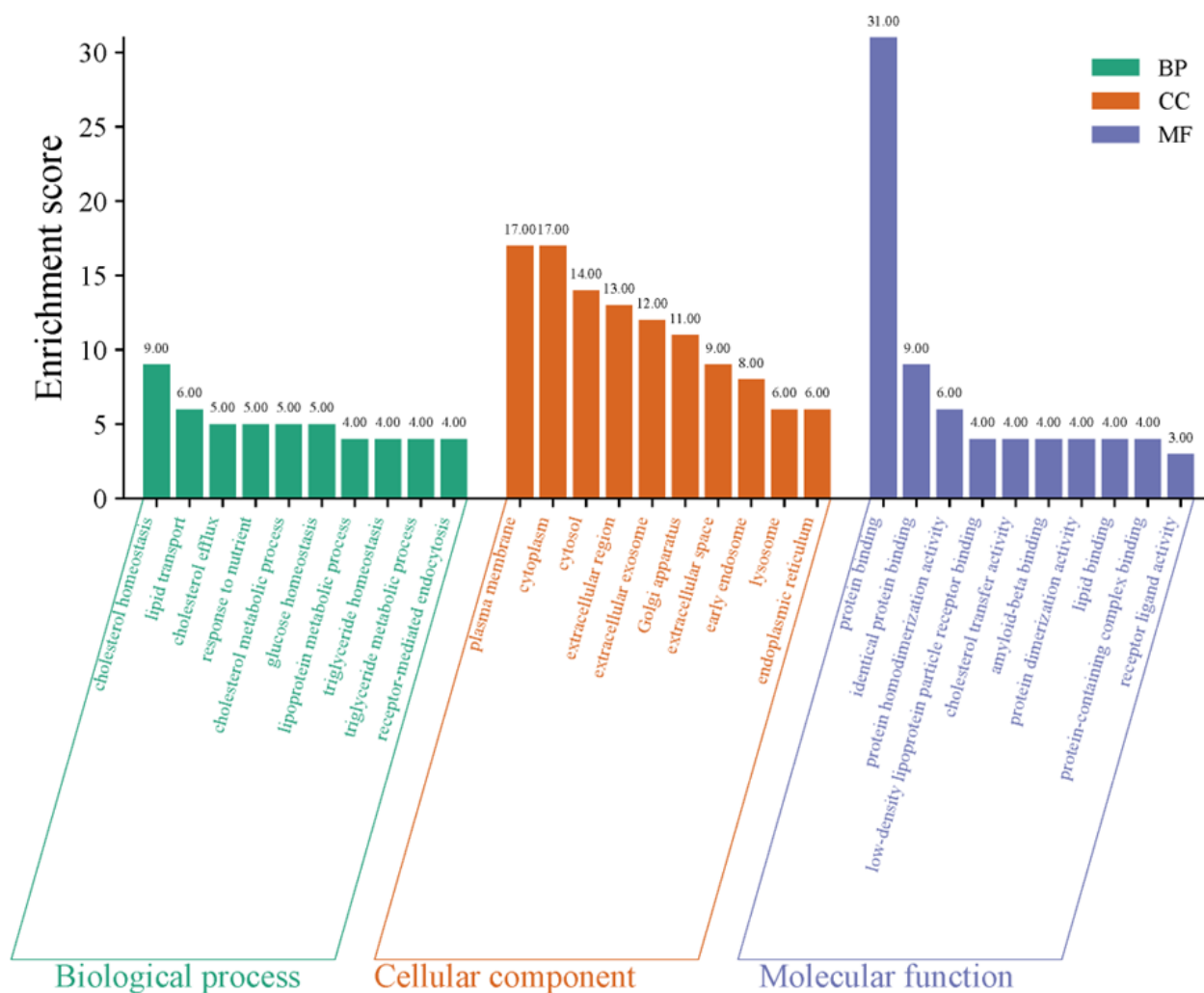
6) GO and KEGG Pathway Enrichment Analysis:

On conducting the Gene Ontology (GO) annotation and KEGG pathway enrichment analysis using a set of potential diabetes-related target genes uploaded to the DAVID 2021 The threshold for identifying significant pathways or gene functions was set P0.05. Fr analysis focused on pathways or gene functions with the highest occurrence count. The GO annotation analysis offered insights into the functional categorization of the genes under study, based on three primary aspects, cellular component (CC), molecular function (MF), and biological process (BP). For cellular components, the analysis is useful to identify the subcellular locations or structures where the genes are predominantly active or present. This information is crucial for understanding the spatial organization and localization of the gene products within the cell Regarding molecular function, the analysis highlighted the specific biochemical activities or

roles performed by the gene products, such as enzyme activity, receptor binding, or response functions. This provided valuable information about the functional properties associated with these genes. The biological process category offered insights into the broader biological events or pathway's in which the genes are involved, including processes like insulin signaling, glucose metabolism, cell cycle regulation, and immune response. Understanding these biological processes is essential for comprehending the roles of these genes in the overall functioning of the organism. The KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway analysis identified seven pathways associated with the set of genes under investigation, as shown in (Figure 5.3) KEGG pathways provide curated information about various biological processes, including cellular signaling, metabolism, and disease-related pathways. In this, the following pathways were significantly associated with the genes: Metabolic pathways, Pathways in cancer. Neuroactive

ligand-receptor interaction, PI3K-Akt signalling pathway, Calcium signaling pathway Alzheimer disease, Chemical carcinogenesis - reactive oxygen species, Serotonergic synapse, Proteoglycans in cancer. The top genes associated with these pathways PYGL, PPARA, ACTN4,

CHRN5, LEPER, NPHS1, NOS3, CFHR1 ABCY3, and CYP19A1 which are know play crucial roles in the regulation of glucose and lipid metabolism, insulin sensitivity, and the overall pathophysiology of Hyperlipidaemia.



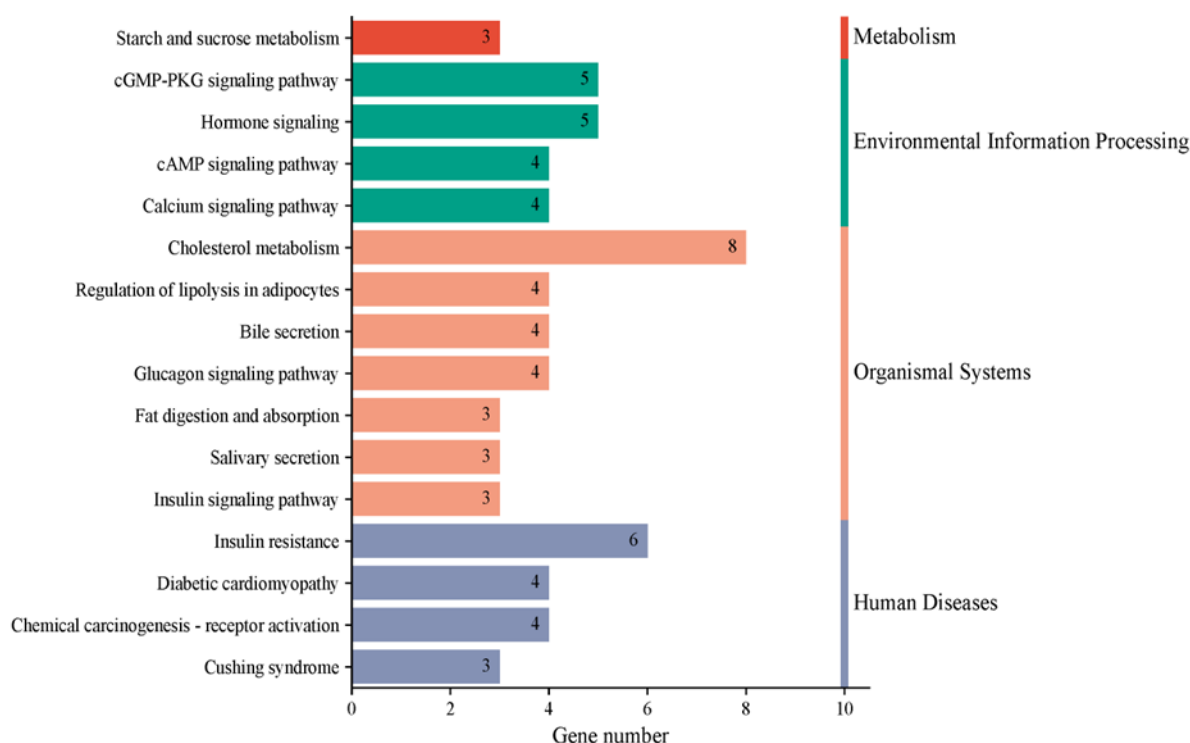


Figure 5.5: (A) Biological Processes [BP], (B) Cellular Component [CC], (C) Molecular Functions [MF], of target genes through the GO enrichment analyses, (D) The KEGG pathway analysis of the target genes: Graphical representation of Gene Ontology classification, highlighting functional annotations and visualization of KEGG pathway interactions, mapping molecular networks and processes.

6.2. Molecular Docking:

To find the active phytoconstituents in *Nigella Sativa* that bind to the PPARA and PYGL receptor, a molecular docking research was conducted. Additionally, to suggest compounds that exhibit promising outcomes as possible therapeutic candidates by looking at their pharmacokinetics and toxicity characteristics. AutoDock vina (1.2.0) was used to determine the interaction between phytoconstituents from the *Nigella Sativa* plant and receptors. Total phytoconstituents were docked, and their binding energies were shown in (Table 6.2), (Table 6.3).

Table 6.2: Binding affinity of different phytoconstituents from *Nigella Sativa* with PPARA

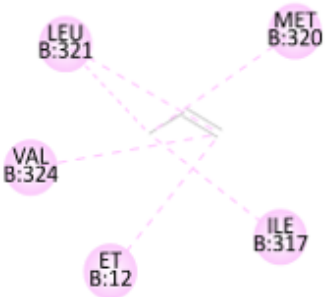
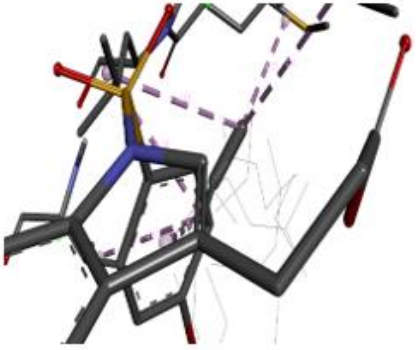
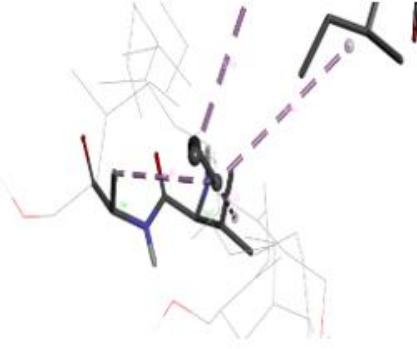
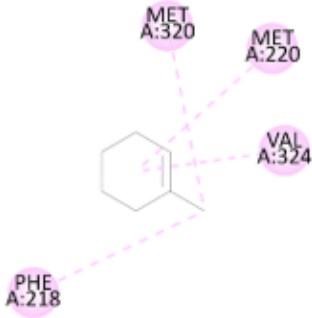
Sr.no	Ligand + Receptor	Binding Affinity (kcal/mol)
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1.	Fenofibrate + PPARA (Standard Drug)	6.9
2.	(E,Z)-Farnesol + PPARA	5.4
3.	Farnesol + PPARA	5.5
4.	(+)-trans-Piperitenol + PPARA	5.7

Table 6.3: Binding affinity of different phytoconstituents from *Nigella Sativa* with PYGL

Sr.no	Ligand + Receptor	Binding Affinity (kcal/mol)
1.	Fenofibrate + PYGL (Standard Drug)	5.2
2.	(E,Z)-Farnesol + PYGL	7.5
3.	Farnesol + PYGL	4.9
4.	(+)-trans-Piperitenol + PYGL	5.1

Table1: Docking structure after interaction between ligand and target protein. (PPAR α)

Phytoconstituent	2D Structure	3D Structure
(E,Z)-Farnesol		
Farnesol		
(+)-trans-Piperitenol		

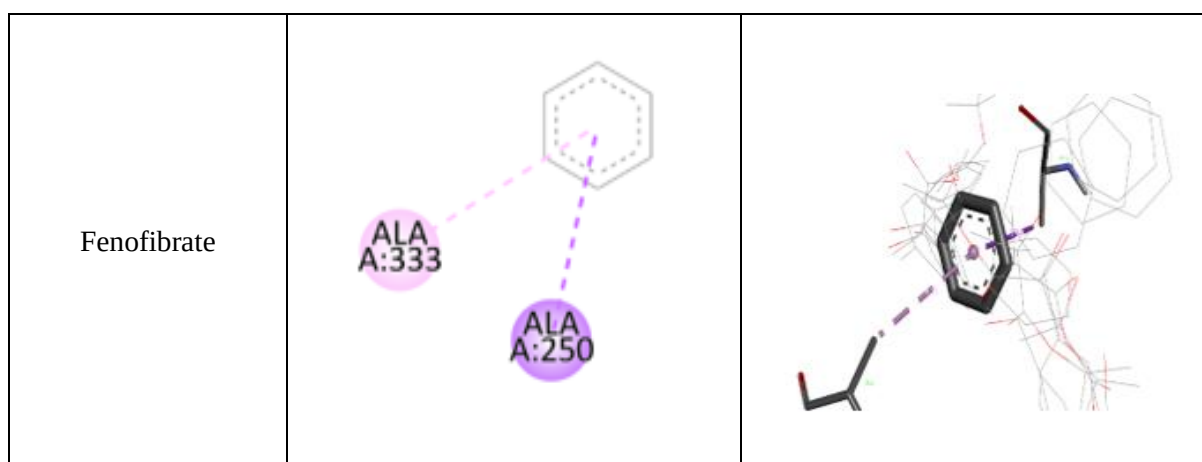
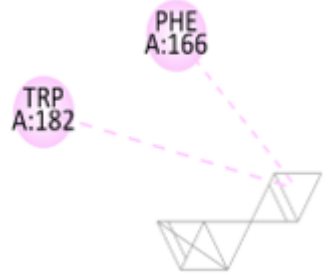
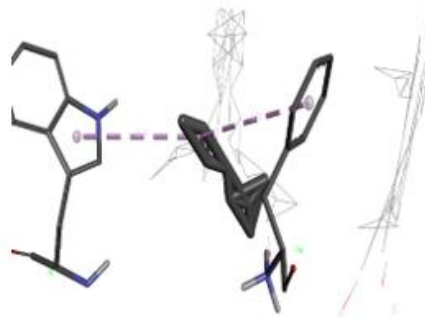
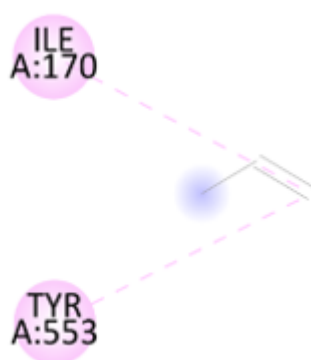
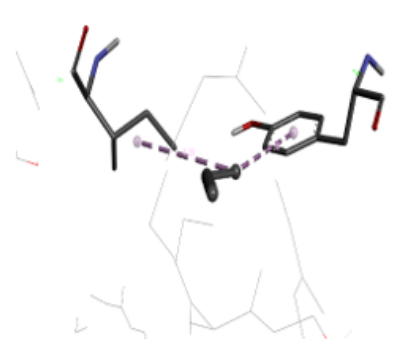
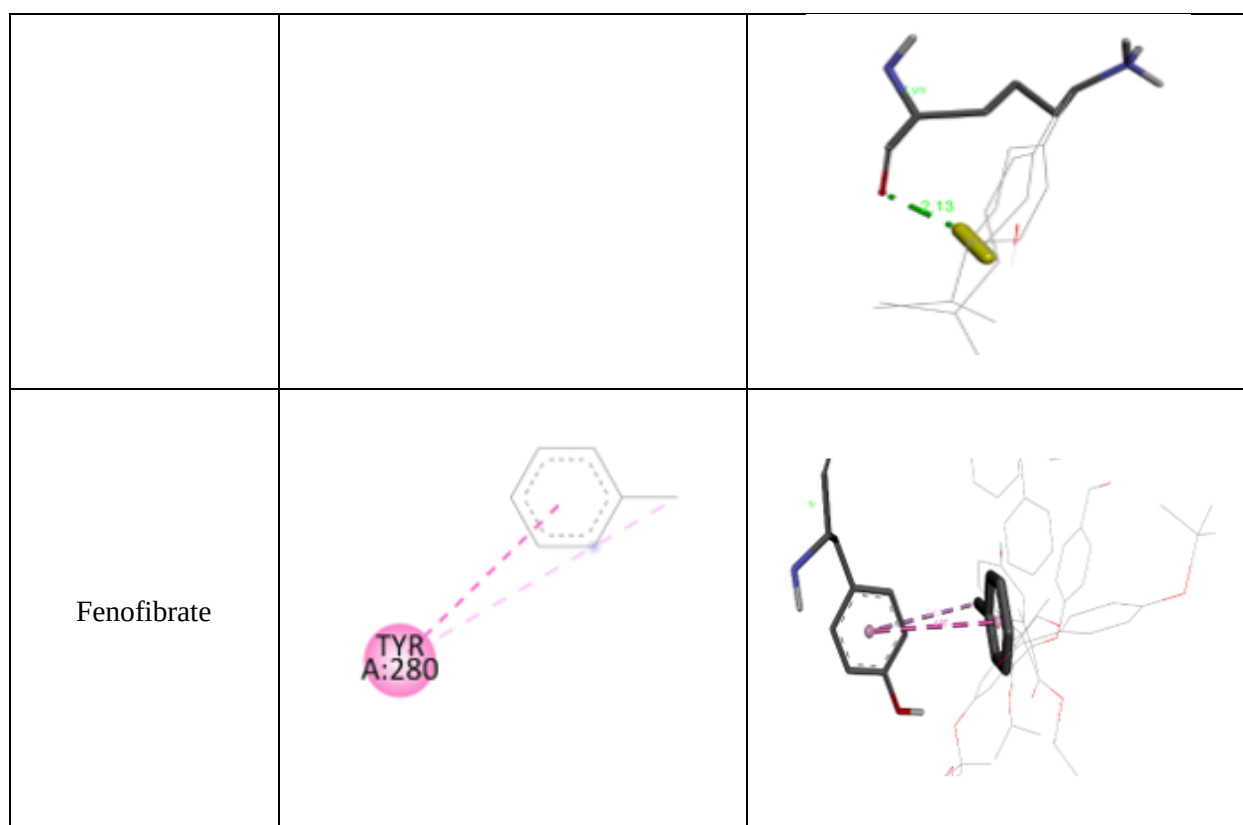


Table1: Docking structure after interaction between ligand and target protein. (PYGL)

Phytoconstituent	2D Structure	3D Structure
(E,Z)-Farnesol	 2D docking structure of (E,Z)-Farnesol showing interactions with PHE A:166 and TRP A:182.	 3D docking structure of (E,Z)-Farnesol showing interactions with PHE A:166 and TRP A:182.
Farnesol	 2D docking structure of Farnesol showing interactions with ILE A:170 and TYR A:553.	 3D docking structure of Farnesol showing interactions with ILE A:170 and TYR A:553.
(+)-trans-Piperitenol		

Table 2: Virtual screening of *Nigellia Sativa* Phytoconstituent against PPAR α

Sr.no	Chemical constituent	Binding affinity with PPAR α	Bonding	Amino acid with numbering	Bond distance
1	Fenofibrate	6.9	1) Pi-sigma 2) Pi-alkyl	1) ALA: A333 2) ALA: A250	4.11 3.74
2	(E,Z)-Farnesol	5.4	1) Alkyl 2) Pi-alkyl	1) LEU: B321 2) VAL: B324 3) ET: B12 4) ILE: B317 5) MET: B320	5.15 5.20 4.28 5.17 4.27
3	Farnesol	5.5	1) Alkyl	1) ASP: A369 2) ASP: A371 3) LYS: A370 4) VAL: A490	3.36 5.44 4.39 4.46
4	(+)-trans-Piperitenol	5.7	1) Alkyl 2) Pi-alkyl	1) MET: A320 2) MET: A220 3) VAL: A324 4) PHE: A218	3.89 5.16 5.41 4.55

Table 2: Virtual screening of *Nigellia Sativa* Phytoconstituent against PYGL

Sr.no	Chemical constituent	Binding affinity with PYGL	Bonding	Amino acid with numbering	Bond distance
1	Fenofibrate	5.2	1) Pi-Pi T-shaped 2) Pi-alkyl	1) TYR: A280	5.12

2	(E,Z)-Farnesol	7.5	1) Pi-alkyl	1) TRP: A182 2) PHE: A166	5.15 5.02
3	Farnesol	4.9	1) Alkyl 2) Pi-alkyl	1) ILE: A170 2) TYR: A553	5.28 5.38
4	(+)-trans-Piperitenol	5.1	1) Alkyl	1) LYS	2.13

Table: - ADME profile of *Nigella Sativa* Phytoconstituents

Sr. No.	Phyto-constituent	Lipinski rule of five		BBB	GI absorption	Log p
		H-bond acceptor	H-bond donor			
1.	(E, Z)-Farnesol	1	1	Yes	High	-3.81
2.	Farnesol	1	1	Yes	High	-3.81
3.	(+)-trans-Piperitenol	1	1	Yes	High	-5.73

The table presents the ADME (Absorption, Distribution, Metabolism, and Excretion) profile of selected phytoconstituents found in *Nigella sativa*, focusing on key drug-likeness parameters. The analysis includes compounds such as (E,Z)-Farnesol, Farnesol, and (+)-trans-Piperitenol. Each compound was evaluated according to **Lipinski's Rule of Five**, which is commonly used to predict oral bioavailability. All three compounds satisfy two primary Lipinski criteria, having only one hydrogen bond acceptor and one hydrogen bond donor each, indicating a favorable molecular structure for drug development. In terms of distribution properties, all three phytoconstituents show positive blood-brain barrier (BBB) permeability, suggesting their potential to reach

the central nervous system if administered systemically. Furthermore, gastrointestinal (GI) absorption is reported as high for each compound, indicating good oral absorption potential. The log P values, which reflect lipophilicity, differ slightly among the compounds: both (E,Z)-Farnesol and Farnesol have a log P of **-3.81**, indicating moderate hydrophilicity, whereas (+)-trans-Piperitenol has a lower log P of **-5.73**, suggesting higher polarity and potentially less membrane permeability compared to the others. Overall, these ADME parameters suggest that the *Nigella sativa* phytoconstituents evaluated in this study possess promising drug-like characteristics, particularly in terms of oral bioavailability and central nervous system accessibility.

Table: Toxicity profile of *Nigella sativa* phytoconstituents

SR.NO.	Phyto-constituent	Hepatotoxicity	Neurotoxicity	Carcinogenicity	Mutagenicity	Cytotoxicity
1.	(E, Z)-Farnesol	Inactive	Inactive	Inactive	Inactive	Inactive
2.	Farnesol	Inactive	Inactive	Inactive	Inactive	Inactive
3.	(+)-trans-Piperitenol	Inactive	Inactive	Inactive	Inactive	Inactive

The table summarizes the toxicity profiles of three phytoconstituents derived from *Nigella sativa*: (E,Z)-Farnesol, Farnesol, and (+)-trans-Piperitenol. These compounds were evaluated across five key toxicological parameters: hepatotoxicity, neurotoxicity, carcinogenicity, mutagenicity, and cytotoxicity. Remarkably, all three phytochemicals were classified as inactive in each of these categories. This indicates that these compounds do not exhibit toxic effects on the liver (hepatotoxicity) or the nervous system (neurotoxicity), nor do they pose risks of cancer development (carcinogenicity), genetic mutation (mutagenicity), or cell damage (cytotoxicity) under the conditions tested. Such an outcome is significant as it supports the safety profile of these natural constituents, suggesting that they could be considered safe candidates for further pharmaceutical or therapeutic development. These findings, when combined with favorable ADME (Absorption, Distribution, Metabolism, and Excretion) profiles, position (E,Z)-Farnesol, Farnesol, and (+)-trans-Piperitenol as non-toxic and biocompatible compounds suitable for future drug development initiatives.

DISCUSSION

Hyperlipidemia remains a critical risk factor for cardiovascular diseases and metabolic disorders. In this study, a comprehensive in silico approach was employed to explore the anti-hyperlipidemic potential of *Nigella sativa*, commonly known as black seed. Using network pharmacology, ADMET screening, and molecular docking, the study identifies key phytoconstituents of *N. sativa* with promising interactions against targets involved in lipid metabolism and hyperlipidemia. From the 192 phytochemicals initially identified in *N. sativa*, only five were directly linked to hyperlipidemia-related targets. Network pharmacology analysis revealed 37 common genes

involved in the pathogenesis of hyperlipidemia, including PPARA, PYGL, NOS3, LEP, CFHR1, and ACTN4. These genes are known to be integral to lipid metabolism, energy regulation, inflammation, and vascular homeostasis, highlighting the potential of *N. sativa* in modulating multiple biological pathways associated with dyslipidemia. The protein-protein interaction (PPI) network, constructed using STRING and visualized in Cytoscape, demonstrated high interconnectedness among the target proteins (clustering coefficient: 0.69), suggesting cooperative activity in biological processes. The top hub genes identified—such as PPARA and PYGL—serve as central regulatory nodes, making them critical targets for therapeutic intervention. Molecular docking studies further validated these targets. Three phytoconstituents—(E,Z)-Farnesol, Farnesol, and (+)-trans-Piperitenol—exhibited strong binding affinities with PPARA and PYGL. Notably, (E,Z)-Farnesol demonstrated the highest binding affinity with PYGL (-7.5 kcal/mol), surpassing even the standard reference drug, fenofibrate (-5.2 kcal/mol). These interactions occurred at key amino acid residues known to influence lipid metabolism. The pharmacokinetic evaluation (ADME profile) of these compounds revealed high gastrointestinal absorption, blood-brain barrier (BBB) permeability, and favorable Lipinski Rule of Five compliance, indicating their drug-likeness and oral bioavailability. Furthermore, the toxicity assessment confirmed that all selected phytoconstituents were non-toxic, exhibiting no hepatotoxic, neurotoxic, mutagenic, or carcinogenic effects, enhancing their suitability as safe drug candidates. The GO and KEGG pathway enrichment analyses identified that these compounds are involved in regulating critical biological pathways, such as PI3K-Akt signaling, insulin regulation, lipid metabolism, and calcium signaling. These pathways are known to play a



major role in the pathophysiology of hyperlipidemia and its associated complications such as diabetes, atherosclerosis, and cardiovascular diseases. Taken together, the results demonstrate that *Nigella sativa* exhibits promising multi-targeted therapeutic potential against hyperlipidemia. The identified compounds not only bind effectively to key targets but also meet safety and drug-likeness criteria, supporting their future development as plant-based antihyperlipidemic agents.

CONCLUSION

The present study employed a comprehensive computational approach—combining network pharmacology, molecular docking, and ADMET analysis—to explore the therapeutic potential of *Nigella sativa* (black seed) for the treatment of hyperlipidemia. Out of 192 phytoconstituents initially identified from *N. sativa*, three key compounds—(E,Z)-Farnesol, Farnesol, and (+)-trans-Piperitenol—emerged as promising candidates based on their strong interactions with crucial target proteins involved in lipid metabolism, particularly PPARA and PYGL. Network pharmacology analysis revealed 37 genes shared between hyperlipidemia-related targets and *N. sativa* compounds, indicating a strong multi-targeted potential. Further, pathway enrichment studies demonstrated that these genes are involved in significant biological processes such as lipid metabolism, PI3K-Akt signaling, insulin response, and calcium signaling, all of which play pivotal roles in the pathophysiology of hyperlipidemia and related cardiovascular and metabolic disorders. Molecular docking confirmed the high binding affinities of the selected phytoconstituents, particularly (E,Z)-Farnesol, which showed a better binding score against PYGL than the standard drug fenofibrate. In addition, pharmacokinetic (ADME) and toxicity (ADMET) profiling

confirmed that these compounds possess high oral bioavailability, blood-brain barrier permeability, and no signs of hepatotoxicity, carcinogenicity, or mutagenicity. In conclusion, this study supports the multi-target, non-toxic, and pharmacologically viable nature of *Nigella sativa* constituents as natural therapeutic agents for the management of hyperlipidemia. These findings provide a valuable scientific foundation for further experimental validation and the potential development of plant-based antihyperlipidemic drugs.

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