Biological Forum – An International Journal **15(2): 246-255(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

A Molecular Docking Study of Indirubin against Hyperlipidemia

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(Received: 23 December 2022; Revised: 01 February 2023; Accepted: 06 February 2023; Published: 09 February 2023)
(Published by Research Trend)

ABSTRACT: A known risk factor for cardiovascular disease is hyperlipidemia. Herbal therapy for hyperlipidemia is popular because it has fewer adverse effects, is less costly, and is readily available. Several medicinal herbs have been shown in studies to lower blood cholesterol levels by decreasing the action of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA). However, conducting direct studies in animals and humans to evaluate the efficacy of herbal medicines for hyperlipidemia raises ethical concerns. Therefore, in silico studies are needed to evaluate the safety and efficacy of phytoconstituents before animal studies and human clinical trials are conducted. This problem can be solved by using insilico studies. Molecular docking has become increasingly important as a method for drug discovery. Molecular docking can be used to mimic the interaction between a small molecule and a protein at the atomic level. This allows us to define the behaviour of small molecules at the binding sites of target proteins and shed light on important biochemical processes. Molecular docking plays an important role in the search for new drugs at significantly lower cost and faster pace. Aim: The aim of study is to evaluate natural products against hyperlipidemia using molecular docking. Material and Natural products were selected after reading various literature sources. For each chemical, a molecular structure file was obtained from the PubChem database. The crystal structure of the protein (PDB ID: 1HW9) was obtained from the Protein Data Bank. The protein molecule was freed from all bound components (ligands and cofactors) and solvent molecules. Active binding sites were identified using the Biovia Discovery programme. PyRx was used to perform docking experiments for natural products against the 1HW9 protein. Results of molecular docking study shows that indirubin has a more negative binding energy value than atorvastatin and it binds more strongly to the receptor HMG-CoA. In this study, numerous bioactive compounds were screened using the Lipinski five rule, and indirubin was found to be more stable toward HMG-CoA than atorvastatin. It can be concluded that the indirubin has the potential to act as an antihyperlipidemic drug.

Keywords: Dyslipidemia, Molecular Docking, Bioactive, Hyperlipidemia**,** Indirubin.

INTRODUCTION

Hyperlipidemia is a condition characterized by increased levels of lipids, such as cholesterol and triglycerides, in the blood. Hyperlipidemia is a medical disorder characterized by an increase in one or more plasma lipids, plasma lipoproteins, and low-density lipoproteins, and a decrease in high-density lipoproteins (Ramkumar *et al.*, 2016). One of the most important risk factors for cardiovascular disease is an increase in plasma lipids. This can lead to an increased risk of cardiovascular disease and other health problems. Recent studies have shown that the prevalence of hyperlipidemia continues to increase. Estimates suggest that up to one-third of adults in the United States have elevated cholesterol. This increase is likely due to a combination of factors, including unhealthy dietary habits, physical inactivity, and obesity. In 2022, a study found that hyperlipidemia was a significant independent risk factor for developing cardiovascular disease in people with type 2 diabetes. The study, which involved more than 8,000 individuals with type 2 diabetes, found that individuals with hyperlipidemia had a significantly higher risk of cardiovascular disease than individuals with normal lipid levels (González-Lleó *et al.,* 2022). A systematic review of studies from 2022 found that hyperlipidemia occurs in nearly 20% of the population worldwide (Liu *et al.,* 2022). Further research is needed to understand the mechanisms underlying this condition and to develop new and more effective treatments. Statins are a commonly used drug to treat hyperlipidemia because they lower cholesterol levels by inhibiting an enzyme called HMG-CoA reductase, which is involved in cholesterol synthesis. Statins are associated with a number of side effects, including muscle weakness, liver damage and increased risk of diabetes. Meanwhile, statins and fibrates continue to be used as the main antihyperlipidemic drugs for the treatment of elevated plasma cholesterol and triglycerides, respectively, but at the cost of significant muscle and liver side effects (Shattat, 2014).

Reducing hyperlipidemia, limiting disease progression, and ameliorating the effects of atherosclerosis are the main goals of atherosclerosis treatment (Khan *et al.*,

2021). Atherosclerosis has increased in morbidity and death in recent years as the common pathological basis of numerous cardiovascular diseases. Unfortunately, there are still numerous problems in the treatment of AS, and the prevention and treatment of the disease are not optimal. Previous research has shown that flavonoids from herbal remedies are common secondary metabolites. Flavonoids have antioxidant activity in diseases due to the many active hydroxyl groups in their structure (Grijalva-Guiza *et al.,* 2021). Plant molecules, on the other hand, have been shown to be effective in lowering blood lipid levels and may be a safer alternative to statins. An example of a plant molecule with lipid-lowering properties is plant sterols, which are found in a variety of foods such as nuts, grains, and vegetable oils. In addition to their lipid-lowering effects, plant molecules have been shown to have other potential health benefits. For example, plant sterols are associated with a lower risk of certain cancers and may have anti-inflammatory properties (Jones and AbuMweis 2009). The role of plant products in the treatment of hyperlipidemia has been the subject of numerous studies in recent years. A systematic review published in 2022 found that diets rich in plant foods such as fruits, vegetables, whole grains, nuts, and seeds were associated with lower levels of total cholesterol, LDL cholesterol, and triglycerides (Yokoyama *et al.*, 2017). Plant foods have also been found to have lipid-lowering effects. For example, the polyphenols found in green tea have been found to lower cholesterol levels (Liu *et al.*, 2022). Tangeretin lowers cholesterol through regulating the Liver X receptor alpha-angiopoietin-like 3-lipoprotein lipase pathway, making it a promising phytochemical for the prevention or treatment of dyslipidemia (Chen *et al.,* 2021). There are several medicinal herbs for daily use. The antihyperlipidemic effect of medicinal herbs is of great importance in reducing obesity diseases, which are the most common cause of death worldwide. The lipid-lowering effects of medicinal plants are currently being investigated worldwide for phytomedicine research and drug development for such diseases. The antihyperlipidemic effect of the plant is helpful in reducing heart disease. As a result, there is growing interest in natural lipid-lowering therapies (Koriem, 2014). Plant sterols have been shown to lower cholesterol levels by inhibiting the absorption of cholesterol in the intestine (Gylling *et al.,* 2014). Another plant molecule with potential for treating hyperlipidemia is policosanol, a mixture of long-chain alcohols found in sugarcane (Gouni-Berthold and Berthold 2002). Policosanol has been shown to lower LDL cholesterol and increase HDL cholesterol, leading to an improvement in the lipid profile in people with hyperlipidemia (Berthold *et al.*, 2006). A flavonoid called quercetin, found in apples, onions, and other foods, significantly lowered cholesterol and triglyceride levels in mice fed a high-fat diet (Su *et al.,* 2022). Polyphenols from grapes and red wine lowered cholesterol levels in humans (Lupoli *et al.,* 2020). In addition to their potential to lower cholesterol, plant-derived molecules may have other cardiovascular health benefits. For example, a plant-based diet high in flavonoids has been associated with a lower risk of cardiovascular disease (Trautwein and McKay 2020). Flaxseed and barley-rich diet formulations significantly enhanced high-density lipoprotein levels in rats while decreasing total cholesterol, triglycerides, and low- and very low-density lipoprotein levels. Of the four distinct combinations studied, the barley-rich combination decreased cholesterol the most efficiently. Phytochemical research also revealed the presence of alkaloids, carbohydrates, proteins, steroids, and cardiac glycosides.

While further research is needed to fully understand the mechanisms by which plant molecules may affect hyperlipidemia and to determine the optimal doses and duration of treatment, these results suggest that plant molecules may represent a promising alternative or adjunct to the treatment of hyperlipidemia. Molecular docking is a technique used to transfer the three-dimensional, computer-generated structure of a smaller medicinal molecule to a protein structure in a variety of orientations, conformations, and positions. This approach is important for drug discovery and medicinal chemistry because it provides information about molecular recognition. Docking is increasingly used to search vast chemical libraries for new drugs (Jakhar *et al.,* 2020). One of the key techniques in current drug design is molecular docking, which forecasts whether a protein-ligand or protein-protein interaction will result in a stable protein-ligand complex. The protein-ligand complexes have been facilitating a variety of biological functions. Vander Waals forces and the creation of hydrogen bonds allow the ligands to attach to proteins (Maryshyla and Nevaditha 2020). Molecular docking is a computational method for predicting the binding orientation and affinity of small molecules, such as drugs, to a specific protein target. This is important because it can aid in the discovery and development of new drugs by predicting which compounds are likely to bind to a target protein and how strongly they will bind. This can help researchers identify potential drug candidates and optimize their properties before they are synthesized and tested in the lab (Pinzi and Rastelli 2019). In addition, molecular docking can be used to understand how a small molecule affects the function of a protein and to aid in the rational development of new drugs to treat specific diseases (Torres *et al.,* 2019). Molecular docking is widely used in the pharmaceutical industry as an in silico approach to identify potential drug candidates. It is also used in the field of computational drug discovery to screen large compound libraries against a target protein, providing a cost-effective and efficient way to identify promising lead structures (Meng *et al.*, 2011). The study of complicated biological and chemical systems and the development of new drugs both greatly benefit from the use of molecular docking. The goal of the docking approach is to examine the ligand conformation or

experimental binding affinities of macromolecular targets inside the binding site. Lead compound optimization and virtual screening investigations to discover new physiologically active compounds are conducted using conventional computational and experimental drug design methodologies. Fundamentally, the sampling techniques and scoring functions used in docking methodology are used to generate and assess ligand poses. Docking is a well-known approach for accelerating the drug design process since it is simple to grasp in terms of structure-function correlations, automated docking, and virtual screening. Obesity arises when the body's energy intake exceeds its energy consumption on a consistent basis, and pharmacologically boosting the activity of brown adipose tissue and browning of white adipose tissue has been regarded as potential techniques to treat obesity. Indirubin may have a protective impact in the prevention and treatment of obesity and its problems since it is an effective brown adipose tissue (as well as beige cell) activator. Obesity is frequently associated with problems in lipid metabolism. Indirubin protects against obesity caused by a high-fat diet (HFD). Tumor necrosis factor-α, interleukin-6, and monocyte chemoattractant protein-1 cytokines were less expressed in mouse liver tissues following indirubin administration under HFD. In HFD-fed animals, indirubin therapy improves glucose metabolism, decreases lipid accumulation in adipose tissue and adipocyte size, and decreases hepatic fat deposition. Indirubin may be a promising medicine for treating obesity and obesity-related diseases due to its ability to induce Uncoupling protein 1 (UCP1) expression and improve mitochondrial respiratory efficiency in vitro. Brown adipose tissue contains UCP1. Indirubin is a promising natural product for the prevention of obesity and related diseases by increasing brown adipose tissue activity and inducing browning of subcutaneous inguinal white adipose tissue, at least in part by activating the Protein Kinase A (PKA) and p38 Mitogen-activated Protein Kinase (MAPK) signal pathways. Indirubin, a pure chemical derived from the indigo plant, reduces body weight and adiposity while improving glucose homeostasis and insulin sensitivity in HFD-fed rats. In vitro investigations indicated that indirubin could increase the expression of thermogenic genes in differentiated brown adipocytes. Indirubin has the ability to directly activate brown adipocytes in a cell-autonomous manner. High mitochondrial DNA copy numbers and high rates of oxygen consumption are two significant properties of brown adipose tissue or beige adipocytes. Indirubin administration dramatically increased the mitochondrial oxygen consumption rate in differentiated brown adipocytes, indicating an increase in mitochondrial activity. Indirubin can stimulate brown adipocyte activity by enhancing mitochondrial activity and upregulating brown adipose tissue -enriched gene expression. In HFD mice, indirubin therapy significantly decreased body weight gain and adiposity while improving whole-body metabolism, but there was no significant change in normal chow diet settings. In vivo indirubin administration led to greater UCP1 expression levels in brown adipose tissue and subcutaneous inguinal white adipose tissue, which was consistent with the in vitro screening results. Indirubin administration also significantly increased the expression of brown adipose tissue -enriched thermogenic genes involved in lipid metabolism and mitochondrial biogenesis, as well as the oxidative phosphorylation proteins in brown adipose tissue and subcutaneous inguinal white adipose tissue. Importantly, indirubin therapy browns subcutaneous inguinal white adipose tissue, as seen by increased expression of beige-specific markers in HFD-fed animals. Indirubin balanced body weight and fat mass, which were likely implicated in brown adipose tissue activation and subcutaneous inguinal white adipose tissue browning, as well as enhanced energy expenditure and thermogenic gene expression in brown adipose tissue and subcutaneous inguinal white adipose tissue. Indirubin administration had no noticeable impact on the expression levels of common adipogenic creator genes (Peroxisome proliferator-activated receptor 2; Human Adipocyte Fatty Acid-Binding Protein) in brown adipose tissue and subcutaneous inguinal white adipose tissue, but dramatically raised adiponectin in brown adipose tissue. Indirubin can enhance systemic glucose and lipid homeostasis, alleviate hepatic steatosis, and clearly reduce the expression of inflammation-related genes in HFD-induced liver tissues (Wei *et al.*, 2020).

MATERIAL AND METHODS

Ligand preparation. All ligands were retrieved from the PubChem database in standard data format. Marvin View software was used for analysis. The canonical SMILES IDs of the selected ligands were noted, and the physicochemical properties of these ligands were derived using the PubChem database. Subsequently, 3D structures of the selected ligands were generated using Biovia Discovery software (Kavitapu and Sharma, 2021).

Protein preparation. There is no "ideal PDB structure", only the one that best fits the requirements. Different entries often correlate with different bound ligands and often simulate the Michaelis complex or transition state. Others are stuck in certain conformations due to mutations or ligand binding and may have originated from a particular creature, such as Homo sapiens. Thus, PDB ID 1HW9 was selected based on variables such as species, resolution and R factor, protein, length for which the structure is solved, and whether the structure is in native or ligand-bound form. The protein from the Protein Data Bank (PDB ID: 1HW9) was downloaded in PDB format and then refined and purified using Biovia Discovery Studio. All co-crystals, heteroatoms and water molecules were removed from the protein.

Active binding site analysis. Biovia Discovery software was used to detect active binding sites. The

selected protein active binding sites provide information about the restricted region of each protein. In this study, the target proteins and the selected chemicals were docked using a blind technique.

Molecular docking analysis. A number of docking tools are available for virtual screening based on docking. In this study, accessible and user-friendly software called PyRx (https://pyrx.sourceforge.io) is used. Other applications such as AutoDock, AutoDock vina, and Open Babel are all included in PyRx. After docking was completed, the docked proteins were analyzed. The results were then saved and all compounds were ranked based on the docking score (Trott and Olson 2010). Protein-ligand interactions involve the binding of a ligand molecule to a specific site on a protein, resulting in a conformational change of the protein. These interactions play a critical role in numerous biological processes, including enzyme catalysis, signal transduction, and regulation of gene expression (Du *et al.,* 2016). An example of a protein-ligand interaction is the interaction between the protein receptor and its ligand, a molecule that activates the receptor. Receptor-ligand interactions are essential for signal transduction in cells. In this process, a signaling molecule binds to a specific receptor on the surface of a cell, resulting in a conformational change of the receptor that triggers downstream signaling pathways (Heldin *et al.,* 2016). In addition to specific interactions between a protein and its ligand, there are also nonspecific interactions such as hydrophobic interactions, van der Waals forces, and hydrogen bonding that can also contribute to the binding affinity of a protein-ligand complex (Frutiger *et al*., 2021). Overall, protein-ligand interactions play an important role in many biological processes and are the focus of much drug discovery research, as targeting these interactions can lead to the development of new therapeutic agents (Ballante, 2018). Atorvastatin is a statin drug used to lower cholesterol levels in the body. It works by inhibiting the enzyme HMG-CoA reductase, which is responsible for the synthesis of cholesterol. Binding of atorvastatin to HMG-CoA reductase occurs through several interactions with specific amino acid residues in the enzyme. Atorvastatin was found to bind to residues Val522, Cys527, Met534, Ile762, Gln814, and Cys817 of the HMG-CoA reductase enzyme. A binding interaction of atorvastatin with the 1HW9 amino acids occurs via hydrophobic interactions. Atorvastatin contains a large hydrophobic region capable of interacting with the hydrophobic residues of the enzyme, such as valine and isoleucine. These interactions help stabilize the binding of atorvastatin to the enzyme and prevent it from being readily detached. Another binding interaction of atorvastatin with the 1HW9 amino acids occurs via hydrogen bonds. Atorvastatin contains several polar groups, such as amides and alcohols that are capable of forming hydrogen bonds with the polar residues of the enzyme. These hydrogen bonds help to further stabilize the binding of atorvastatin to the enzyme and increase its affinity for the enzyme. In addition to the hydrophobic and hydrogen bonding interactions, atorvastatin can also interact with 1HW9 amino acids through van der Waals forces. These forces are weaker than the other binding interactions, but still contribute to the overall stability of the atorvastatin-enzyme complex. Overall, several interactions with specific amino acid residues in the enzyme are involved in the binding of atorvastatin to HMG-CoA reductase, including hydrophobic forces, hydrogen bonding, and van der Waals forces. These interactions help stabilize the complex and increase the affinity of atorvastatin for the enzyme, allowing it to effectively inhibit cholesterol synthesis in the body. These binding interactions are critical to the effectiveness of atorvastatin as a cholesterol-lowering drug. Indirubin is a small molecule that binds to various amino acids in proteins, as shown in Fig. 5. The binding of indirubin to HMG-CoA reductase involves a variety of interactions with specific amino acid residues in the enzyme, such as hydrogen bonding and van der Waals forces. The overall binding interactions demonstrate the versatility of indirubin in its ability to bind to a variety of amino acids and regulate protein activity.

RESULTS AND DISCUSSION

HMG-CoA is essential for controlling the cholesterol biosynthesis pathway (Baskaran *et al.,* 2015; Haber *et al.,* 2013). Inhibiting HMG-CoA reductase activity reduces cholesterol production and hence increases hepatic absorption of low-density lipids (LDL) via LDL receptor regulation (Marahatha *et al*., 2021). Taking statins as an HMG-CoA reductase inhibitor in humans is linked with a number of side effects, including hepatotoxicity, myopathy, gastrointestinal discomfort, cataracts, rhabdomyolysis, and an increased risk of diabetes (Ramkumar *et al.,* 2016). Dietary elements are important components used in traditional medicine in India, and they may have the ability to inhibit many enzymes and scavenge free radicals (Nguyen *et al*., 2019; Tanwar *et al*., 2018). Furthermore, thanks to evidence-based investigations of their safety and effectiveness, herbal formulations based on complementary medicine are garnering increased attention and recognition in India and many other countries. Many herbal compositions for the treatment of hypercholesterolemia and diabetes have been documented in the literature, based on traditional knowledge passed down from generation to generation (Moss and Ramji 2016; Patil *et al*., 2009; Tanwar *et al.,* 2018; Yin *et al.,* 2008). Dietary antioxidant formulations are also effective in reducing risk factors linked with hypercholesterolemia (Mahdavi *et al.,* 2020; Modak *et al.,* 2007).

Multiple studies have indicated that employing plant-derived antioxidant formulations rather than manufactured medicines to prevent oxidative harm is preferable (Alamu *et al.,* 2021; Marahatha *et al.,* 2021). A number of phytoconstituents have also been found to have substantial antioxidant effects (Forni *et al.,* 2019;

Zhang *et al*., 2015). Phytoconstituents have been described as "revitalized medication" and "a basis of dietary antioxidants" (Goutam, 2017b; Mahmoud *et al*., 2019). In addition, locals in rural regions employ plant-based products based on traditional knowledge to treat a variety of ailments. Dysregulation of the cholesterol biosynthesis pathway and subsequent events are linked to hypercholesterolemia and atherosclerosis (Prasad and Lee 2007). The HMG-CoA reductase enzyme is a critical enzyme in the cholesterol biosynthesis pathway that occurs before the first stable product; hence, it is regarded as a rate-limiting step. As a result, statins (HMG-CoA reductase inhibitors) are used as the primary treatment for hypercholesterolemia. The extract therapy reduced overall cholesterol levels as well as intermediate fractionates like low- and high-density lipoprotein and triglycerides significantly. The excessive formation of free radicals in cells causes oxidative stress, which leads to a variety of metabolic illnesses and animal models that have a negative impact on quality of life. Secondary metabolites found in plants are powerful antioxidants. Including these plants and their phytoconstituents into the human diet can help avoid oxidative stress in cells and tissues while also promoting normal physiology (Lee *et al.,* 2017; Tungmunnithum *et al.,* 2018). As recorded in numerous ancient medicinal systems, different phytoconstituents have variable antioxidant capacity levels, and these secondary metabolites should be regarded as a useful resource in human diets (Grover *et al*., 2002; Johar *et al.,* 2018).

According to a preliminary study, all compounds were docked to HMG-CoA reductase. Compared with atorvastatin, indirubin was found to have a stable ligand-receptor complex. The binding affinity of indirubin with protein 1HW9 was found to be - 8.4 kcal/mol. The binding affinity of atorvastatin (+ve control) with protein 1HW9 was determined to be - 7.8 kcal/mol (Table 1). These two drugs have high binding affinity (Table 2). Indirubin is a natural pigment found in certain plants and animals. It belongs to the indigo family of compounds and has been shown to have a number of biological activities, including anti-inflammatory, antitumor, and antioxidant properties (Yang *et al.*, 2022). Indirubin, the purple component of the blue dye indigo, is extracted from plants such as *Indigofera tinctoria* and *Polygonum tinctorium* Lour. In addition to the leaves of *Isatis tinctoria* and *Polygonum tinctorium*, indirubin has also been found in *Strobilanthes cusia*. Indirubin, a stable isomer of indigo, is a 3,2'-bisindole. The active ingredient of the traditional Chinese drug "Danggui Longui Wan", which has potent activity against myeloid leukaemia, is indirubin (Hu *et al.,* 2015). Indirubin is a pharmacologically significant isomer of blue indigo found in indigo-containing plants. Indirubin is a red dye that is an isomer of both blue and brown indigo. It is the second most important component of indigo naturalis after indigo. Indirubin has a bright red hue that results from spontaneous dimerization between the colourless precursors indoxyl and isatin. Indirubin, like indigo, is only slightly soluble. The chemical formula of this compound is $C_{16}H_{10}N_2O_2$ and its molecular weight is 262.26 g mol À1. Indirubin is rarely used in textile dyeing due to its limited colour fastness. It was discovered that this chemical has anti-cancer properties in animal cancer cells. The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor that is involved in the regulation of lipid metabolism and inflammation. Cholesterol secretion was shown to be significantly decreased in human cells following AHR activation. Studies have shown that indirubin can activate the AhR pathway, leading to changes in lipid metabolism and a reduction in inflammation (Tanos *et al.,* 2012).

Indirubin has been shown to have antioxidant and anti-inflammatory properties, which are also important factors in the development of hyperlipidemia. One way that indirubin may help combat lipid disorders is by reducing inflammation. Inflammation is a key contributor to the development of cardiovascular disease, and indirubin has been shown to have anti-inflammatory effects (Lai *et al.,* 2017). Additionally, indirubin has been shown to have antioxidant properties, which can help protect against the damage caused by free radicals. Free radicals can damage cells and contribute to the development of various diseases, including cardiovascular disease. Oxidative stress and inflammation can contribute to the development of atherosclerosis, which is a buildup of plaque in the arteries that can lead to heart disease and stroke. By reducing oxidative stress and inflammation, indirubin may help prevent the development of atherosclerosis and its complications (Qi *et al.,* 2017).

Indirubin is a natural compound found in the roots of the indigo plant, and it has been studied for its potential pharmacological effects on various diseases, including lipid disorders. In traditional Chinese medicine, this species is said to be a material that may be utilized to cure inflammations and bacterial infections (Yang *et al.*, 2022). In addition, indirubin has been found to modulate the expression of genes involved in lipid metabolism, including peroxisome proliferator-activated receptor (PPAR) alpha and PPAR-gamma. PPARs are nuclear receptors that play a critical role in lipid metabolism, and their activation can lead to an increase in lipid catabolism and a decrease in lipid synthesis (Konno *et al.,* 2020).

Indigo derivatives and the preparation Indigo naturalis, which contains the indigo dye, have long been used to treat a variety of diseases, including fevers, various types of inflammation, and carcinomas. Its pharmacological properties were discovered hundreds of years ago and used in traditional Chinese medicine. The observed pharmacological properties support the usefulness of natural dyes in current medical treatment procedures, not only their application in textile dyeing (Stasiak *et al.,* 2014). Overall, indirubin has shown promise as a potential therapeutic agent for lipid disorders, although further research is needed to fully understand its pharmacological effects and potential clinical applications.

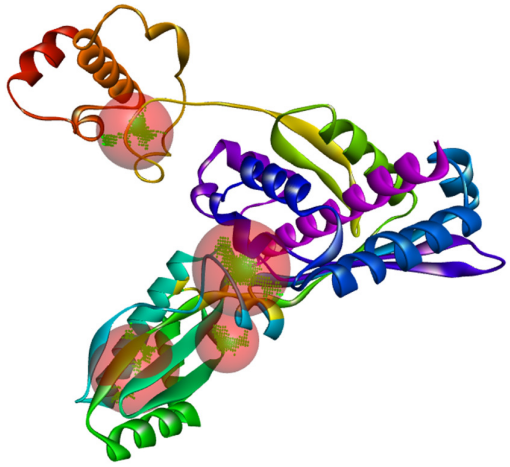


Fig. 1. Protein Molecule (1HW9).

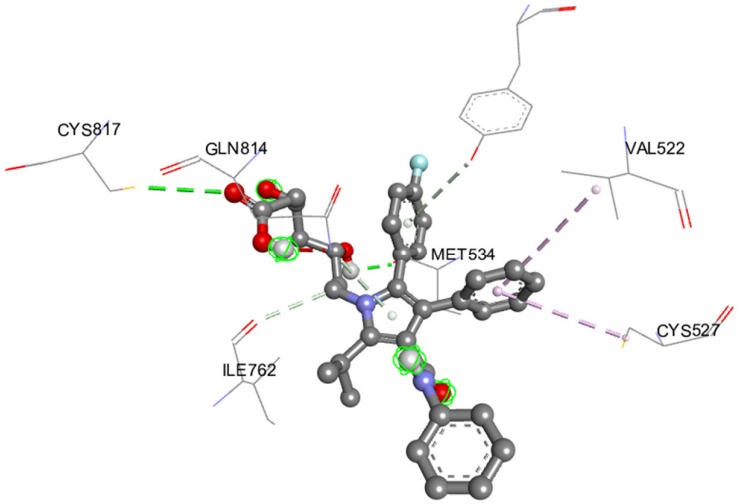
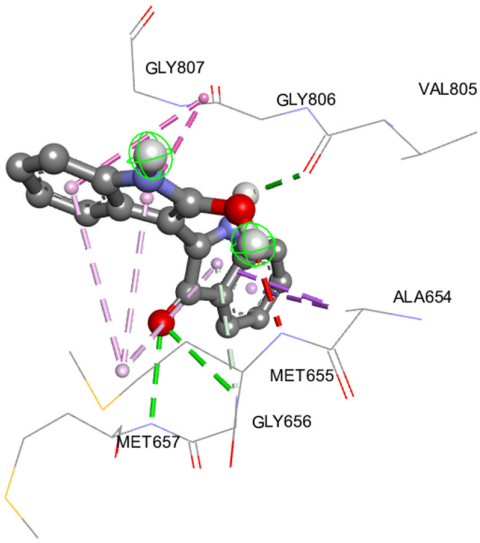
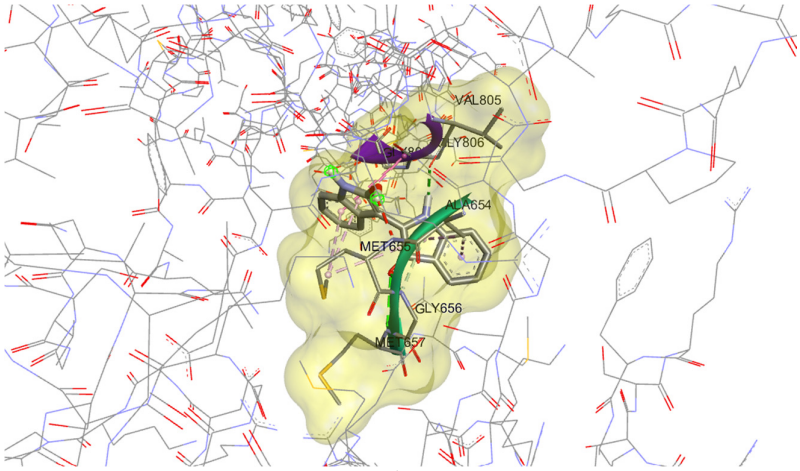


Fig. 2. 3D structure of Atorvastatin (+ve control) docked compound with 1HW9 including amino acids (CYS817, GLN814, ILE762, MET534, CYS527 and VAL522)



(a)



(b)

Fig. 3: (a) & (b) 3D structure of Indirubin docked compound with 1HW9.

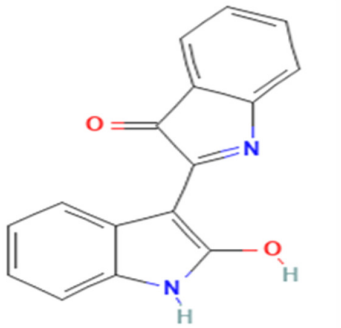


Fig. 4. 2D structure of Indirubin.

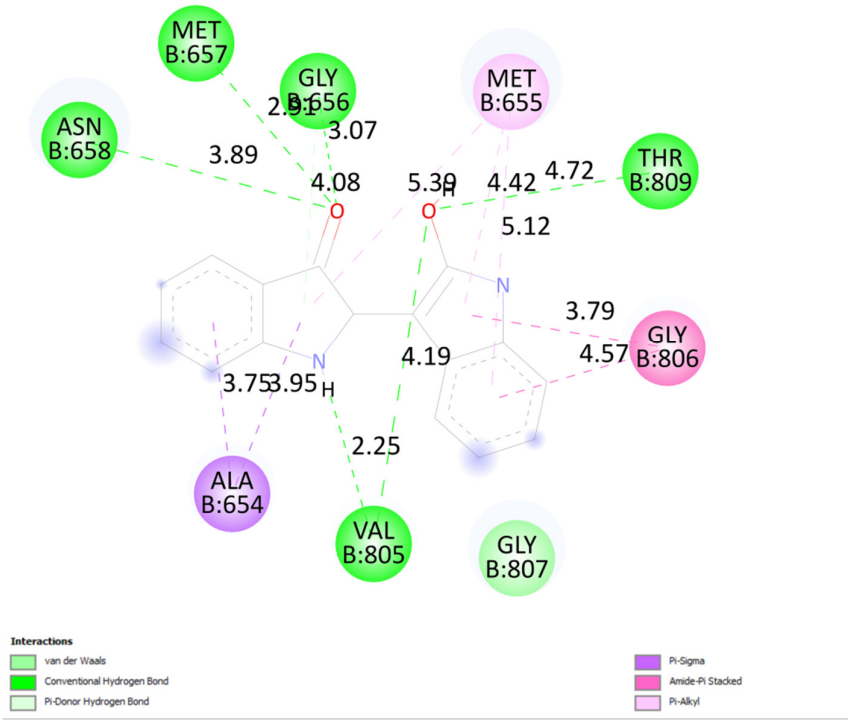


Fig. 5. 2D image of docked pose of Indirubin.

Table 1: Binding Affinity of Indirubin and Atorvastatin.

PubChem ID	Ligand	Protein	Binding Affinity (kcal/mol)
10177	Indirubin	1HW9	-8.4
60823	Atorvastatin (+ve control)		-7.8

Table 2: Comparison of various parameters of Indirubin and Atorvastatin.

Parameters	Indirubin	Atorvastatin
Formula	C16H10N2O2	C33H35FN2O5
Molecular weight	262.26 g/mol	558.64 g/mol
Number of heavy atoms	20	41
Number of aromatic heavy atoms	15	23
Number of rotatable bonds	1	13
Number of H-bond acceptors	3	6
Number of H-bond donors	2	4
Molar Refractivity	80.62	158.26
Topological Polar Surface Area (TPSA)	65.45 Å²	111.79 Å²
Lipophilicity (Log $P_{o/w}$)	1.70	3.48
Solubility	Soluble	Moderately soluble
Gastrointestinal Tract Absorption	High	Low
Druglikeness	Yes	Yes; 1 violation of Lipinski rule : MW>500
Leadlikeness	Yes	No; 3 violations: MW>350, Rotors>7, XLOGP3>3.5

CONCLUSIONS

Currently, hyperlipidemia is a major problem. Conventional treatments can have harmful side effects. This fact has aroused interest in alternative cures, especially in industrialized nations. In nature, there are a variety of healing plants and herbs. More than 200 plants are traditionally used for the prevention and treatment of hyperlipidemia. The relationship between lowering low-density lipoproteins and lower mortality from cardiovascular disease is well known. The lipid-lowering effects of medicinal plants are currently being studied worldwide as part of phytomedicine research for these diseases. However, there are a few herbs that can help people with the aforementioned conditions. The antihyperlipidemic properties of plants are crucial for reducing atherosclerosis. As a result, there is growing interest in natural lipid-lowering therapies. The antihyperlipidemic effects of traditional medicinal plants in many populations are of greater benefit for the development of new drugs to prevent dyslipidemia or atherosclerosis. The bioactive components of herbal medicine may be able to control the multifaceted intervention in lipid metabolism that occurs during the uptake, production, transport, and excretion of cholesterol. The main findings on the use of herbal medicines are positive and suggest potential applications of these drugs in a variety of patient populations. Alkaloids, saponins, polyphenols, and flavonoids are some of the many active ingredients. In addition, the bioactive ingredients in herbal medicines are generally safe and well tolerated. In general, the bioactive constituents of herbal medicines have a variety of mechanisms of action that allow them to alter various metabolic pathways and control plasma lipid levels. Overall, the use of herbal molecules to treat hyperlipidemia offers a potential alternative to statins that may be safer and provide additional health benefits. However, further research is needed to fully understand the mechanisms behind the lipid-lowering effects of these molecules and to determine their optimal use in clinical practice.

FUTURE SCOPE

This study demonstrates the potential of indirubin as a natural product for the treatment of hyperlipidemia. The use of molecular docking has helped identify potential binding sites for indirubin on target proteins, leading to a better understanding of its mechanism of action. Further research is needed to determine the optimal dosage and administration of indirubin for the treatment of hyperlipidemia, but this study offers a promising starting point for the development of new therapies for this disease. Indirubin is a natural product that has been used in traditional Chinese medicine. However, it has poor bioavailability, which means that it may not be absorbed or metabolized effectively in the body. Therefore, the efficacy of indirubin as a drug against hyperlipidemia needs to be evaluated in preclinical and clinical studies.

Acknowledgement. I sincerely thank my Ph.D. advisor for taking good care of me throughout the course of my studies.
Conflict of Interest. None.

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How to cite this article: Anant Kumar Patel (2023). A Molecular Docking Study of Indirubin against Hyperlipidemia. *Biological Forum – An International Journal, 15*(2): 246-255.