IDENTIFICATION OF POTENTIAL PHYTOCHEMICAL CANDIDATES FOR INHIBITION OF NLRP3 INFLAMMASOME IN DCM: AN IN-SILICO STUDY

A DISSERTATION
SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE AWARD OF THE DEGREE
OF
MASTER OF SCIENCE
IN
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Submitted by:

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I Swati Yadav, Roll No. 2K21/MSCBIO/54 student of M.Sc. Biotechnology, hereby declare that the project Dissertation titled "**Identification of potential phytochemical candidates for inhibition of nlrp3 inflammasome in DCM: an in-silico study** "which is submitted by me to the Department of Biotechnology, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Science, is original and not copied from any source without proper citation. This work has not previously formed the basis for the award of any Degree, Diploma Associateship, Fellowship or other similar title or recognition.

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CERTIFICATE

I hereby certify that the Project Dissertation titled "Identification Of Potential Phytochemical Candidates For Inhibition Of NLRP3 Inflammasome In DCM: An In-Silico Study" which is submitted by Swati Yadav, Roll No. 2K21/MSCBIO/54, Department of Biotechnology, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Science, is a record of the project work carried out by the students under my supervision. To the best of my knowledge this work has not been submitted in part or full for any Degree or Diploma to this University or elsewhere.

Yashasanp 02.06.23

Prof. YASHA HASIJA

SUPERVISOR

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Date: 02/06/2023

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ABSTRACT

One of the most prevalent adverse consequences of diabetes is diabetic cardiomyopathy (DCM), which is also the leading cause of death in people with diabetes. This disease was discovered in 1972 and has been the subject of research ever since. Over the past ten years, extensive research has been done on the pathophysiology and clinical characteristics of diabetic cardiomyopathy, but there are currently few proven methods for its prevention and treatment. Basically, DCM occurs because of the unregulated metabolic pathways associated with diabetes, which further result in high oxidative stress along with onset of many inflammatory pathways which can lead to Heart Failure. In some studies, it has been revealed that there are some agents that when focused upon, can be possible cardio protective approaches to prevent the condition. This dissertation goes into detail about a one such agent i.e. NLRP3. Several computational tools, including Autodock Vina, Pymol, Protein Drug Bank, and PILP, are used in this study. (Protein-Interaction Ligand Profiler). By using PILP and molecular docking of the protein and ligand, the interactions between NLRP3 and plant phytochemicals are investigated. Drug discovery is facilitated by research into protein and ligand inter-connection. The purpose of this intense examination is to identify the most effective plant phytochemicals that have demonstrated promise in preventing DCM in humans.

ACKNOWLEDGEMENT

I feel immense pleasure in expressing my deep gratitude and an overwhelming sense of pride towards our guide, Prof. Yasha Hasija, whose constant help and support, complete involvement, invaluable guidance and encouragement, and critical analysis of our result, led us to complete this project and gain preliminary knowledge of research. I owe our sincere thanks to him for giving us an opportunity to work under his guidance.

I would also like to take this opportunity to thank the HOD of the Department of Biotechnology, Prof. Pravir Kumar for overlooking and enabling me to use all the facilities that were required and for going out of his way to help students in any matter.

We would like to express our deepest appreciation to the faculty of Department for all their support, and continuous guidance in laboratory work and research during the project. I express our gratitude toward our families and colleagues for their kind cooperation and encouragement which help us in the completion of this project.

Without the help of fore mentioned individuals the project would not have been as easily carried out, and we would face many difficulties through it.

Swati Yadav

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CHAPTER 1
INTRODUCTION

1.1. Prevailing effects: Diabetes Mellitus

A perennial metabolic condition called diabetes mellitus is characterized by high blood glucose levels. It has serious health ramifications and impacts millions of individuals worldwide. T1D (Type-1 diabetes) and type-2 diabetes (T2D) are two utmost and prevalent subtype of the incurable ailment, while there are other forms as well. While T2D is predominantly characterised by insulin resistance and decreased insulin secretion, T1D is an outcome of autoimmune death of pancreatic beta section cells, leading towards insulin insufficiency. Gestational diabetes and less prevalent monogenic kinds of diabetes are additional types. [1]

Although it can happen at any age, type 1 diabetes, commonly familiar as insulin-dependent diabetes, often progress during childhood or adolescence. It is categorised as an autoimmune condition when body's immunization unintentionally target the self and undergoes degenration of the pancreatic beta cells that assembles to generate insulin. Because of the following circumstance, T1D patients need lifelong insulin therapy to control their blood sugar levels. Though the precise origin of T1D is not yet entirely understood, genetic factors and environmental triggers such viral infections are thought to be involved [2]. Contrarily, type 2 diabetes, which regards for the majority of instances, is the most popular category of the disease. Besides an alarming rise with its frequency among children and adolescents due to the growth in childhood obesity, it predominantly affects adults. Insulin receptivity or resistance, which arises when the cells terminate response towards insulin, and compromised pancreatic insulin output are characteristics of T2D. Obesity, sedentary behaviour, poor diet, ethnicity, family history of diabetes and older age are threatening constituents for T2D. [3]

Traditional diabetes mellitus consequences include macrovascular issues like stroke, coronary heart disease, and peripheral artery diseases inclusive to microvascular issues like retinopathy, diabetic kidney disease, peripheral neuropathy. The Heart failure have considered as prevalent indication towards cardiovascular illness in individual with T2DM (type 2 diabetes mellitus) & it has a sky-high mortality risk [3]. The macrovascular implications of diabetes, similarly

stroke, coronary heart disease & peripheral vascular disease. These are the main genesis of morbidity, mortality and medical costs [4]. Atherosclerotic cardiovascular disease refers to all of these disorders collectively. It's noteworthy that 70% of type 2 diabetes mellitus sufferers who pass away before age 65 do so from cardiovascular disease [5]. The development of cardiac fibrosis, cardiomyocyte hypertrophy, and apoptosis—all of which happen independently of coexisting macrovascular and microvascular diabetes issues—is the distinguishing feature of diabetic cardiomyopathy, a special type of cardiomyopathy to which diabetic people are prone. Observational studies have demonstrated that having diabetes increases a person's chance of getting heart failure by a factor of two to five compared to not having the condition. The pathophysiology of this illness is complicated and poorly understood. [6], [7]

1.2 Foundation to diabetic cardiomyopathy notion

The term "diabetic cardiomyopathy" describes the structural and physiological changes to the heart caused by diabetes mellitus. Diabetes has negative effects on the cardiovascular system in addition to its main effects on blood glucose management. The term "diabetic cardiomyopathy" refers solely to heart changes caused by diabetes alone and not by other recognised causes like hypertension or coronary artery disease. Myocardial hypertrophy (increased heart muscle size), fibrosis (excessive collagen deposition), decreased diastolic function (abnormal heart relaxation), and finally the onset of heart failure are all symptoms of this illness. Because it increases the morbidity and mortality rates linked to diabetes, diabetic cardiomyopathy is a serious problem. [8]

1.3 Significance and relevance of studying DCM

Growing Prevalence: Along with the global rise in diabetes mellitus, DCM incidence is also increasing. Diabetes affects millions of people worldwide, and a sizeable percentage of those people eventually develop cardiac problems like DCM. For the growing problem of diabetes-related cardiovascular disease to be managed, DCM must be understood. [9]

Unique Pathophysiology: DCM differs from other types of cardiac disease in that it has unique pathophysiological pathways. Heart disease is influenced by conventional cardiovascular risk factors like atherosclerosis and hypertension, but DCM involves intricate interactions between hyperglycemia, insulin resistance, inflammation, oxidative stress, mitochondrial dysfunction, and fibrosis. Researching these special systems can help identify new therapy targets and methods that are tailored to DCM [10], [11]

Early Detection and Intervention: Early detection of DCM enables prompt therapies to halt the development of heart dysfunction or limit its progression. By researching DCM, scientists hope to find potential biomarkers, imaging methods, or diagnostic standards that may aid clinicians in spotting the illness in its initial stages. Cardioprotective treatments and managing cardiovascular risk factors are only a few examples of early intervention measures that can enhance outcomes and prevent or delay heart failure in diabetics[11].

Improved Risk Stratification: The goal of DCM research is to pinpoint the variables that influence the onset and evolution of the illness. Clinicians can stratify diabetes patients depending on their risk of developing cardiac problems by identifying the risk variables linked to DCM. This enables more individualised treatment programmes and vigilant monitoring of high-risk patients, optimising healthcare resource allocation and thus lightening the load on healthcare systems [12].

Therapeutic Interventions: Researching DCM offers chances to create specialised treatments and interventions for diabetic heart disease. To stop, slow down, or reverse the progression of DCM, researchers can find prospective pharmacological targets by comprehending the molecular and cellular mechanisms underlying the disease. Pharmacological medications, dietary changes, and interventions aimed at reducing inflammation, oxidative stress, mitochondrial dysfunction, and fibrosis may all be included in these therapies [12], [13].

CHAPTER 2
LITERATURE REVIEW

2.1. DCM's pathophysiology

The electrical conduction system controls the coordinated contraction and relaxation of the heart chambers during normal cardiac activity. This makes it possible for the blood to be pumped and circulated effectively, ensuring that the tissues and organs of the body receive oxygen and nutrients.

Diabetic cardiomyopathy (DCM) is a serious condition that can have a substantial negative effect on the heart. A specific form of cardiac disease called DCM develops as a result of diabetes. Here are several ways that diabetes affects the heart and causes DCM:

Insulin Aversion/ resistance: In subtype-2 diabetes, which is the most common form of the condition, the body becomes resistant to the effects of insulin. Insulin resistance basically leads to high blood sugar levels. These elevated blood glucose levels have the potential to harm blood vessels directly and impede the ability of cardiac cell activity[12].

Chronic Hyperglycemia: Chronic hyperglycemia, or prolonged exposure to elevated blood glucose levels, can out-turns in oxidative stress and inflammation. These processes lead to endothelial dysfunction, which damages the inner lining of blood vessels. Endothelial dysfunction makes it harder for blood vessels to expand and contract appropriately, which affects heart blood flow[12], [13].

Abnormal Glucose Metabolism: Diabetes alters how the heart uses its energy and interferes with the regular metabolism of glucose. Glucose is the main fuel source for the heart's energy requirements. An energy imbalance and decreased cardiac function can result from diabetes because the heart muscle's ability to absorb and use glucose is hampered [14]

AGEs: AGEs (Advanced glycation end products) can develop as a outcome of elevated blood glucose levels in diabetes. Over time, AGEs build up in tissues and organs and are toxic substances. They aid in progression of fibrosis inflammation in the heart, oxidative stress, and resulting in the structural and functional modifications associated with DCM[14].

Oxidative Stress and Inflammation: Oxidative stress is a result of diabetes' promotion of reactive oxygen species generation. Inflammation is a side effect of oxidative stress, which harms cells and tissues, including those in the heart. Fibrosis, which involves excessive collagen deposition and hardening of the cardiac muscle, can be brought on by chronic inflammation in the heart[15], [16].

Microvascular Disease: Diabetes, commonly referred to as microvascular disease, can impact the tiny blood arteries in the heart. Microvascular illness reduces the amount of oxygen and nutrients that reach the heart muscle by obstructing blood flow there. This may result in cardiac muscle cell injury and myocardial ischemia (insufficient blood flow to the heart)[15].

The development of diabetic cardiomyopathy is influenced by a number of diabetes-related variables, including insulin resistance, persistent hyperglycemia, aberrant glucose metabolism, AGEs, oxidative stress, inflammation, and microvascular dysfunction. Heart alterations, such as myocardial hypertrophy, fibrosis, reduced diastolic function, and ultimately heart failure, are characteristics of DCM. Understanding these pathways is essential for creating specialised treatments and management strategies for DCM in diabetics.

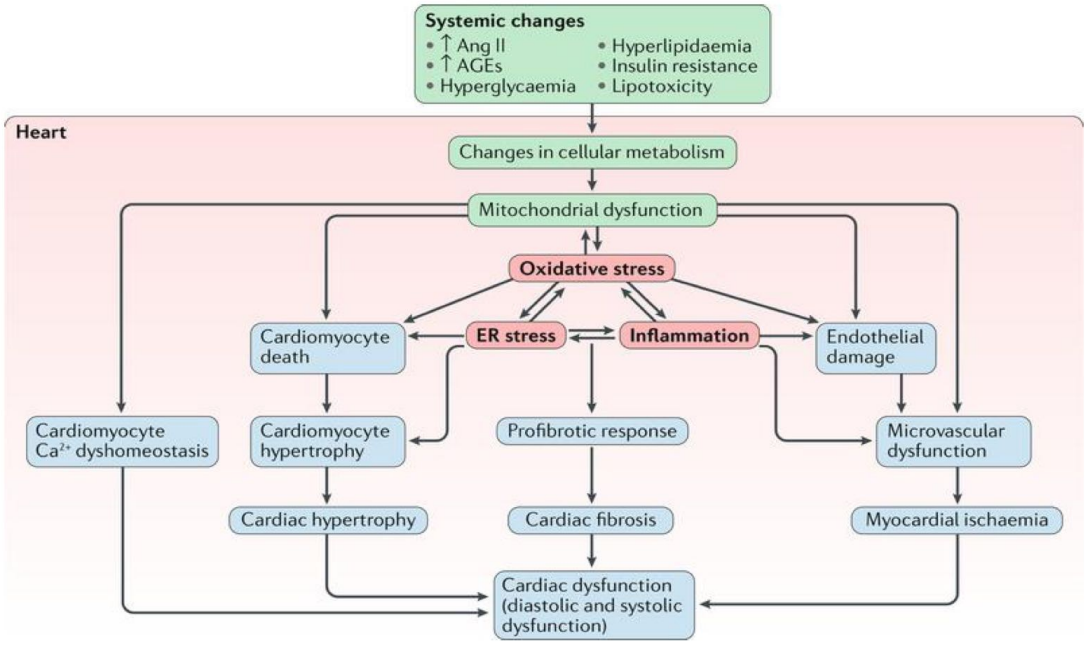


Figure1. Multiple factors involved in the progression of DCM

2.2 Factors affecting DCM

A. Lipotoxicity

The level of free fatty acids (FFA) rises as a consequence of lipotoxicity. FFA activates the peroxisome proliferator-activated receptor (PPAR), increasing absorption via CD36. This, in turn, encourages the activation of several genes involved in lipid metabolism and may cause mitochondrial malfunction [17].

B. Cardiac Dysfunction

A rise in reactive oxygen and nitrogen species (ROS) and a decrease in soluble guanylate cyclase (sGC) activity and cyclic GMP (cGMP) levels are found as a result of lipotoxicity, glucotoxicity, and angiotensin II, which leads to cardiomyocyte stiffness. Additionally, these modifications cause the inflammatory cells that secrete Transforming Growth Factor (TGF) to become active. As a result of this TGF's interaction with its receptor and activation of cardiac fibroblasts, hypertrophy and contractile dysfunction are finally produced. [18]

C. Oxidative Stress

Advanced glycation end products (AGEs) cause (NADPH) oxidase activity to increase, which in turn causes levels of oxidative stress and inflammation to rise. They also cause the molecules of collagen to cross-link. Collagen becomes less elastic as a result of AGEs, which leads to contractile failure. High glucose levels result in the suppression of the following Nrf2- and Sirt1-mediated antioxidant signalling & progressive stimulation of Nf-mediated inflammatory signalling. The generation of ROS and inflammatory factors rises as a result of these alterations[19].

D. Inflammation

High mobility group protein, which can connect with lipopolysaccharides and activate TLR4 on cardiac cells, can be activated by AGEs and Ang II, which can cause cardiomyocyte hypertrophy. In addition, active inflammatory cells like macrophages have the ability to secrete cytokines like tumour necrosis factor (TNF), which can impair contractility[20].

2.3 Progression of DCM

Over time, the heart undergoes a number of structural and functional alterations as diabetic cardiomyopathy (DCM) progresses[21]. Here is a rough outline of the stages and salient characteristics of DCM, even though the precise progression may differ from person to person:

Early Stage: The heart may experience minor alterations in the early stages of DCM that are not yet clinically evident. Metabolic problems such insulin resistance, hyperglycemia, and mitochondrial dysfunction are present throughout this phase. These elements contribute to the heart muscle's oxidative stress, inflammation, and cellular damage. [22]

Structural Changes: Structure alterations become increasingly obvious as DCM develops. Cardiac hypertrophy, or the expansion of heart muscle cells, can happen as a protective reaction to heightened stress and workload. However, excessive collagen deposition, or fibrosis, frequently coexists with this hypertrophy in the heart tissue. Diastolic dysfunction can be caused by fibrosis, which can make the heart muscle rigid and less able to relax during diastole[23].

Impaired Diastolic Function: Diastolic dysfunction is a defining characteristic of DCM. It refers to the heart's diminished capacity to properly relax and fill during the cardiac cycle's resting stage. Normal diastolic relaxation is interfered with by increased myocardial stiffness brought on by fibrosis and abnormal calcium management. Because of reduced blood flow efficiency and increased pressures inside the heart chambers, this can cause symptoms including exhaustion and shortness of breath[24].

Systolic Dysfunction: Systolic dysfunction may manifest in DCM's latter phases. Systolic dysfunction describes the heart's diminished capacity to contract and efficiently pump blood. The decrease of the heart's contractile function is a result of cardiomyocyte injury, cellular apoptosis (programmed cell death), and further fibrosis. Due to a decreased ejection fraction (proportion ratio of blood pumped outside the heart with every contraction), in turn causes fatigue, dyspnea, and fluid retention, which are all signs of heart failure[25], [26].

Heart Failure: Unchecked DCM can eventually result in heart failure, a condition in which the heart is unable to adequately supply the body with blood and oxygen. Extreme tiredness,

dyspnea, edoema (fluid retention), a reduced ability for exercise, and an overall reduction in quality of life are some of the signs of heart failure. To manage symptoms and enhance outcomes at this point, measures like medication, lifestyle modifications, and maybe advanced heart failure therapy may be required [4].

Hence, it is significant to consider that a number of alterables, included as the duration and command over diabetes, patient characteristics, and the existence of other comorbidities, might affect how DCM develops. In order to reduce or stop the course of DCM, it is essential to recognise diabetes early, monitor it frequently, and manage it optimally. Managing DCM and enhancing patient outcomes may also be possible with therapies that address the underlying mechanisms, including as lowering oxidative stress, inflammation, and fibrosis[4].

2.4 Contribution of NLRP3 Inflammasome in DCM

The development of DCM and the beginning of inflammation are both dependent on a multiprotein complex known as the NLRP3 inflammasome [27]. A summary of NLRP3's function in DCM is given below:

1. Inflammation Activation: A key characteristic of DCM that speeds the development of heart dysfunction is chronic low-grade inflammation. The NLRP3 inflammasome is activated to initiate and amplify the inflammatory response [27]. The detection of multiple danger signals, such as mitochondrial dysfunction, reactive oxygen species (ROS), and extracellular matrix components, leads to the activation of caspase-1 and subsequent release of pro-inflammatory cytokines like interleukin-1 beta (IL-1 beta) and interleukin-18 (IL-18). These cytokines promote inflammation in a vicious cycle that damages and malfunctions the heart in DCM[28].

2. Oxidative Stress: A characteristic of DCM, oxidative stress is intimately related to NLRP3 inflammasome activation. ROS produced as a result of metabolic imbalances and hyperglycemia can cause NLRP3 inflammasome formation and activation. An optimistic feedback loop within oxidative stress and inflammasome excitation is established when the activated NLRP3 inflammasome causes more ROS generation. In

DCM, this persistent oxidative stress has a role in heart fibrosis, remodelling, and decreased contractile performance[29].

3. Cardiac Remodelling: The unfavourable cardiac remodelling that occurs in DCM results in modifications to the structure and operation of the heart. Excitation of the NLRP3 inflammasome have been inter-linked to the promotion of cardiac fibrosis and hypertrophy, two essential elements of unfavourable remodelling[30]. Extracellular matrix proteins are deposited and fibrotic tissue is formed as a result of the inflammasome being activated, which also causes the release of pro-fibrotic factors and stimulation of fibroblast activation. The progression of DCM is made worse by this remodelling process, which impairs myocardial function[27], [30].

4. Metabolic Dysfunction: Activation of the NLRP3 inflammasome has also been connected to the abnormalities in metabolism seen in DCM, including insulin resistance and dyslipidemia. The inflammasome activation causes metabolic dysfunction and exacerbates cardiac damage in DCM by interfering with insulin signalling pathways, limiting glucose absorption, and encouraging lipid accumulation in cardiomyocytes[31] .

Given the significant role the NLRP3 inflammasome plays in the pathophysiology of DCM, targeting its activation has emerged as a promising therapeutic approach. Numerous research have looked into how to reduce NLRP3 inflammasome activation and lessen the negative consequences of inflammation and oxidative stress in DCM by using NLRP3 inhibitors, anti-inflammatory drugs, and antioxidants.

2.5 NLRP3 Inhibitors that are under study

Investigations into possible NLRP3 inhibitors and their therapeutic uses are still ongoing. Some substances are being researched for their potential utility in treating different inflammatory and cardiovascular illnesses, including DCM, after showing promise in preclinical trials. Here are a few illustrations:

1. MCC950: In preclinical tests, MCC950, a tiny chemical, showed effective and precise suppression of the NLRP3 inflammasome. It has demonstrated effectiveness in a variety of illness models, including those involving inflammatory and cardiovascular conditions. Its precise applicability in DCM, however, is still being looked at[32].

2. OLT1177 (Dapansutrile): OLT1177 is a further NLRP3 inhibitor that has been proven in preclinical investigations to have anti-inflammatory properties. It has been studied in a number of inflammatory illnesses and may be used in cardiovascular diseases. Its application in DCM hasn't been completely investigated yet[33].

3. CY-09: In preclinical trials, this recently discovered small chemical showed inhibitory results on NLRP3 inflammasome activation. In animal replica of inflammatory disorders, it has demonstrated promise in lowering inflammation and tissue damage. Its potential use in DCM, however, warrants more research[34].

OBJECTIVE OF THIS STUDY

Through my study I am identifying potential phytochemical candidates with high binding affinity for the NLRP3 inflammasome

CHAPTER 3
MATERIALS AND METHODOLOGY

3.1. METHODS AND MATERIAL

3.1.1. Phytochemicals

Soybeans and other legumes contain the isoflavone **genistein**. Numerous biological activities, including anti-inflammatory capabilities, are displayed by it. Genistein have been demonstrated to downregulate NLRP3 inflammasome activation and reduce IL-1 production in numerous experimental models. It functions by preventing the NF-B signalling pathway and limiting ROS emission [35].

The flavonoid **luteolin** can be found in a variety of fruits, vegetables, and herbs. It has immunomodulatory, antioxidant, and anti-inflammatory effects. Due to luteolin's inhibitory effects on NLRP3 inflammasome activation, less IL-1 is produced. It affects inflammasome signalling and decreases the progression of NLRP3 and pro-inflammatory cytokines by inhibiting the NF-B pathway [36].

The roots of the Scutellaria baicalensis plant used in traditional Chinese medicine are used to extract a flavonoid known as baicalein. Studies have been done on its anti-inflammatory and antioxidant effects. Baicalein has been found to suppress both the release of IL-1 and the activated catalysis of the NLRP3 inflammasome. By inhibiting NF-B activation and ROS generation, it modifies NLRP3 inflammasome signalling [37].

The phenolic component salidroside is present in the medicinal plant rhodiola rosea. Its anti-inflammatory and antioxidant effects have been researched. In numerous cell and animal models, salidroside has been found to suppress NLRP3 inflammasome activation and decrease IL-1 production. It works by inhibiting the NF-B pathway and lowering ROS production [38].

The flavonoid rutin can be found in a variety of fruits, vegetables, and grains. It demonstrates anti-inflammatory and antioxidant impacts. Rutin have been discovered to constitute inhibitory effects on the acttivating effect of the NLRP3 inflammasome and the generation of IL-1 in experimental experiments. By lowering ROS levels and inhibiting NF-B activation, it modifies inflammasome signalling[39].

The bioactive substance andrographolide is obtained from the plant Andrographis paniculata. It has immunomodulatory and anti-inflammatory effects. According to studies, andrographolide can stop the NLRP3 inflammasome from activating and lessen IL-1 production. It works by reducing NF-B signalling and preventing the activation of caspase-1[40].

Various plants, particularly Berberis species, contain the isoquinoline alkaloid berberine. Its anti-inflammatory and metabolic benefits have been researched. It has been discovered that berberine inhibits both the production of IL-1 and the activation of the NLRP3 inflammasome. It affects inflammasome signalling by reducing ROS generation, inhibiting NF-B activation, and controlling the expression of NLRP3 [41].

3.1.2 Target Protein- NLRP3 Protein

The NLRP3 inflammasome is also known as the "NLR family pyrin domain-containing 3." The abbreviation NLRP3 is made up of the first letters of its full name. The NLR (nucleotide-binding domain and leucine-rich repeat containing) family of proteins, which regulates inflammation and innate immune responses, includes the protein NLRP3. It is made up of the effector protein pro-caspase-1, the adaptor protein NLRP3, and the protein known as ASC (apoptosis-associated speck-like protein with a caspase recruitment domain).

3.1.3 Websites

PDB- PDB is shorthand for Protein Data Bank. The knowledge on 3-D (three-dimensional) structures of natural macromolecules, including nucleic acids, protein and complex assemblies, is available in a sizable and well-known database. Researchers from a variety of disciplines can

access and analyse protein structures in the PDB to learn more about their relationships, functions, and possible applications.
https://www.rcsb.org/

PUBCHEM- The vast chemical compound database PUBCHEM is maintained by the National Centre for Biotechnology Information (NCBI), a section of the National Library of Medicine (NLM) in the United States. It offers details on the biological functions, material characteristics, chemical compositions, and associated information about millions of chemical compounds.
https://pubchem.ncbi.nlm.nih.gov/

PLIP- Protein-Ligand Interaction Profiler is what it's called. It is a programme used to compute, analyse, and display protein-ligand interactions. With the help of PLIP, you may locate and examine binding sites, hydrogen bonds, hydrophobic interactions, and other kinds of interactions that take place between proteins and ligands.
https://plip-tool.biotec.tu-dresden.de/plip-web/plip/index

SwissADME- Absorption, Distribution, Metabolism, and Excretion are the letters in the acronym ADME. It describes the procedures by which a substance is taken in by the body, disseminated to various tissues, metabolised (chemically changed), and then eventually expelled from the body.
A drug's pharmacokinetics and pharmacodynamics are greatly influenced by its ADME characteristics. In order to evaluate a compound's bioavailability, effectiveness, safety, and potential drug-drug interactions, it is crucial to study its ADME properties.
http://www.swissadme.ch/

3.1.4 Softwares

PyMol Visualization Tool , Autodock MGL tool 1.6.3, Autodock Vina 1.4.6

3.2 METHODOLOGY

1. Ligand selection and preparation- 7 Ligand (phytochemicals) were selected from the IMPPAT database- Andrographolide, Baicalein, Berberine, Genistein, Luteolin, Rutin and Salidroside that shows anti-NLRP3 properties.(Fig. 2)

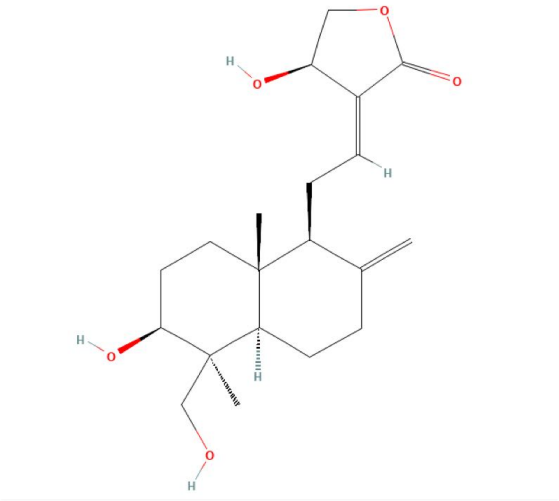
2. These ligands were collected from (https://pubchem.ncbi.nlm.nih.gov/) (SDF, 3D conformer). Further, refined ligands were transformed to pdb format using PyMol software, and then saved in pdbqt format with the Autodock tool 1.6.3, which makes them competent for molecular docking.

3. Protein preparation- 3D structure of Target protein i.e.NLRP3 was selected and retrieved from online protein data bank website (https://www.rcsb.org/structure/) with PDB ID 8ETR . Using Auto dock 1.6.3 tools water molecules were removed and additional polar H and Kolman's charge were added to the protein, which makes it suitable for docking and the structure was saved in pdbqt format.

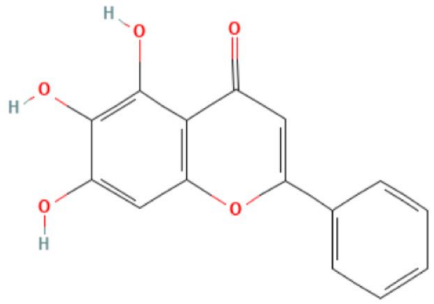
4. Docking in process- Protein and ligand molecular docking is initiated by formatting the grid in Autodock tool with x, y, and z centers in 8.704, 1.183, and 12.215 points, respectively. X, Y, and Z are each 40, 40, 40 in size. Energy range is 4 by default and exhaustiveness of docking is taken 8. Then docking is done by Auto Dock Vina 1.4.6. In auto dock vina software, all phytochemicals (ligands) are docked with protein.
   And we get the binding energy of each ligand with target protein.

5. Analysis: Using the Auto dock vina tool, an output file is created. This output is then run via the PyMol software to create a pdb file, and the results are analyzed using PLIP to see which amino acid of ligand bind to the protein.

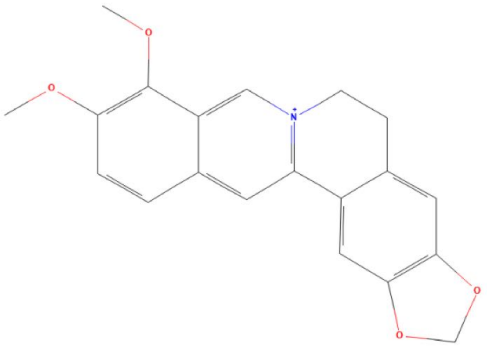
6. ADME assay- Using swissADME one can find out or evaluate the pharmacokinetics parameters which includes Absorption, Distribution, Metabolism and Excretion. This is one of the significant method for the research and drug development. For this test the canonical smiles structures of phytochemicals from PubChem were obtained.



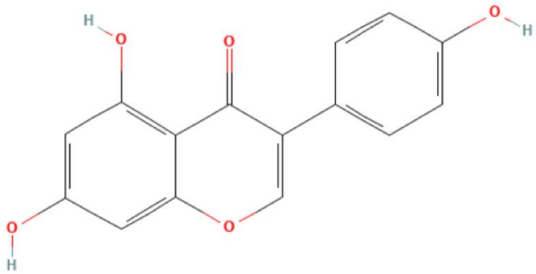
1 Andrographolide



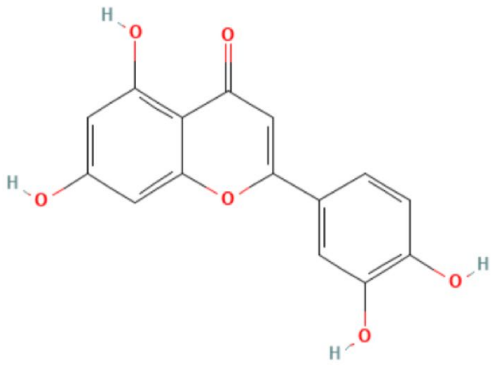
2 Baicalein



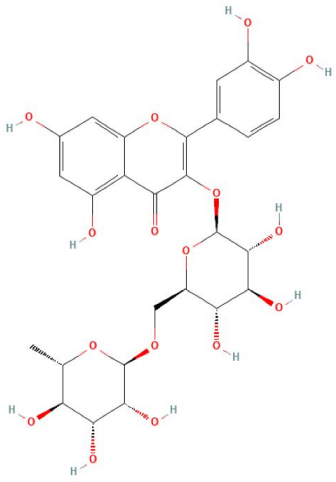
3 Berberine



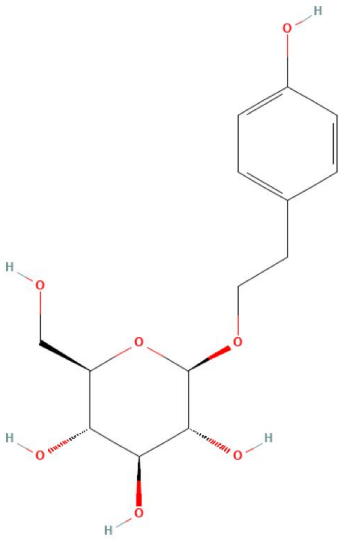
4 Genistein



5 Luteolin



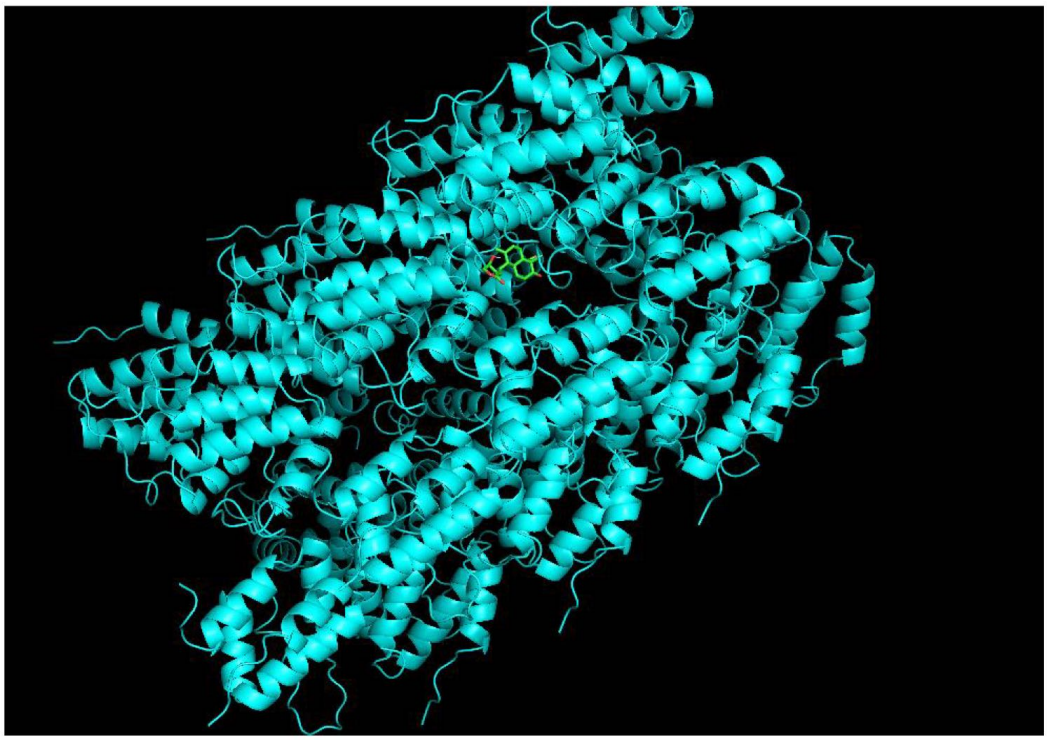
6 Rutin



7 Salidroside

Figure 2: 2D Images of Ligands – 1 Andrographolide, 2 Baicalein, 3 Berberine, 4 Genistein, 5 Luteolin, 6 Rutin and 7 Salidroside

CHAPTER 4- RESULTS



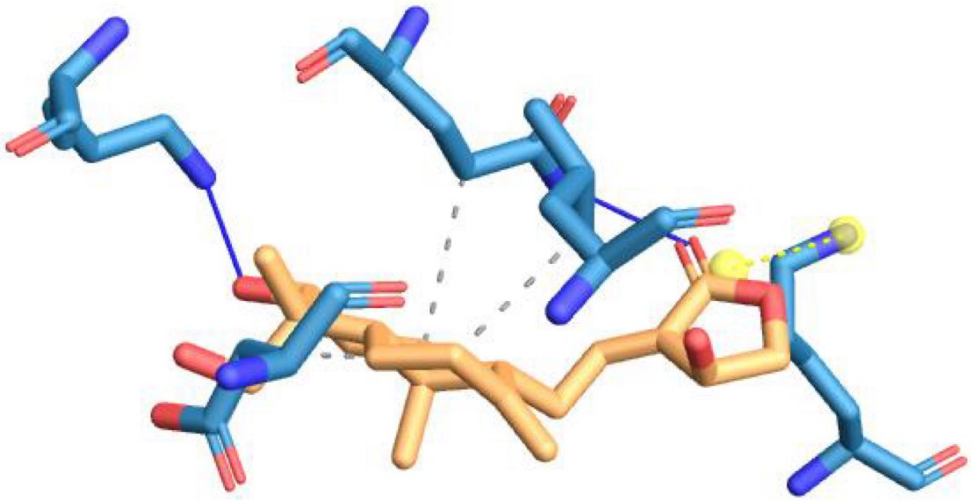
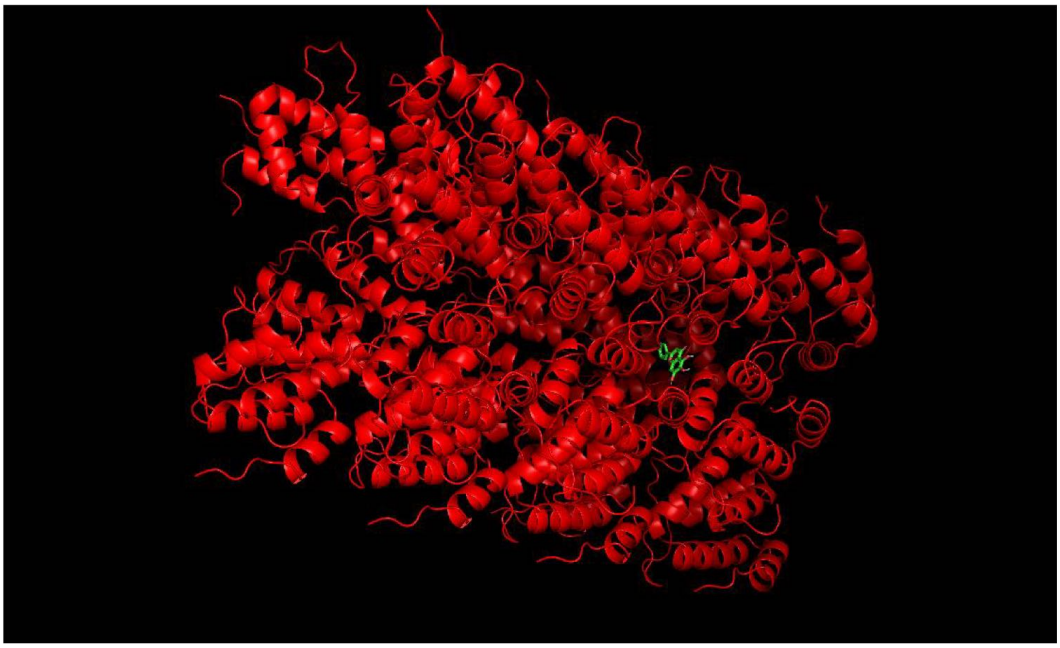


Figure 1. Interaction of Andrographolide with NLRP3 and its PLIP analysis.



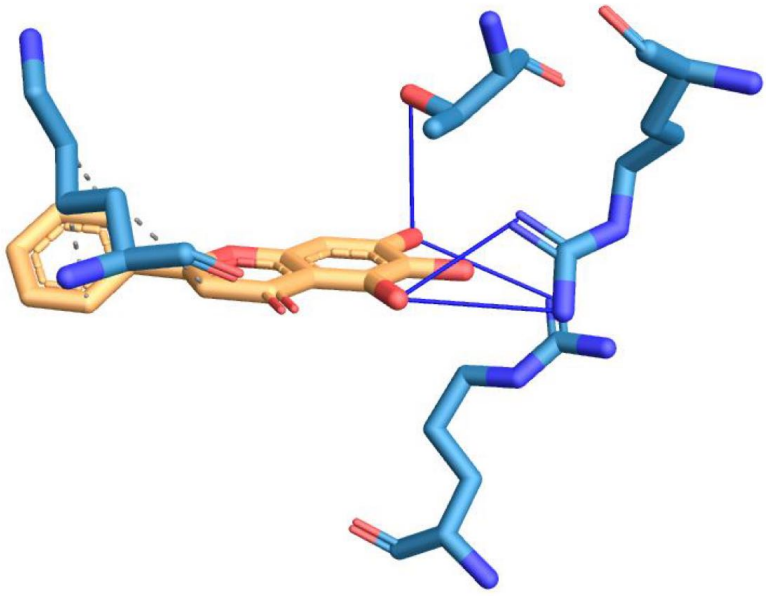
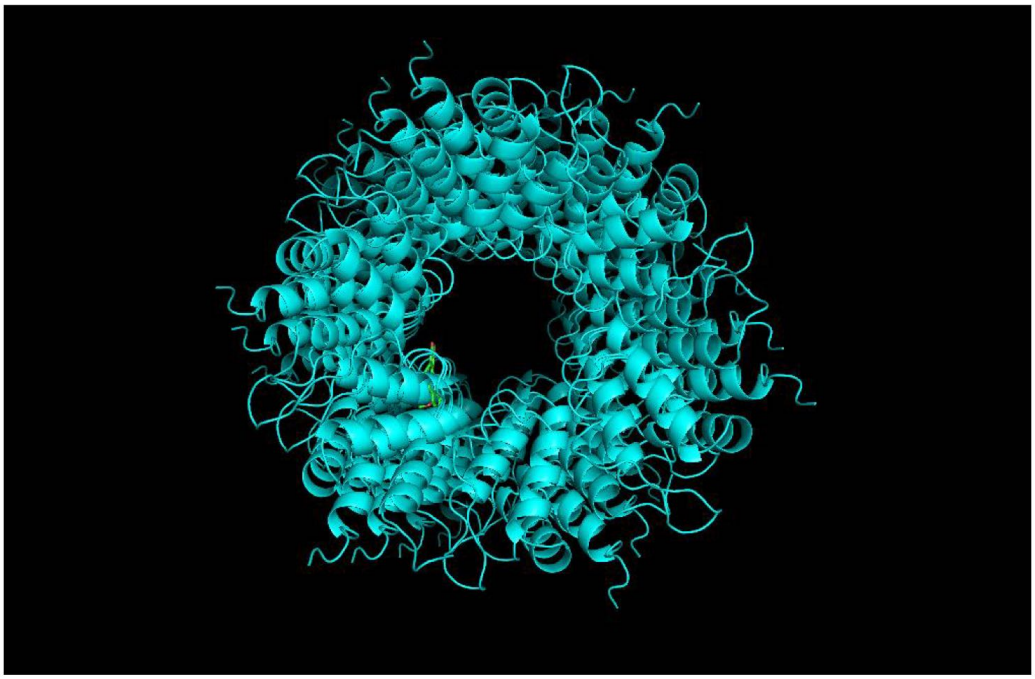


Figure 2. Interaction of Baicalein with NLRP3 and its PLIP analysis.



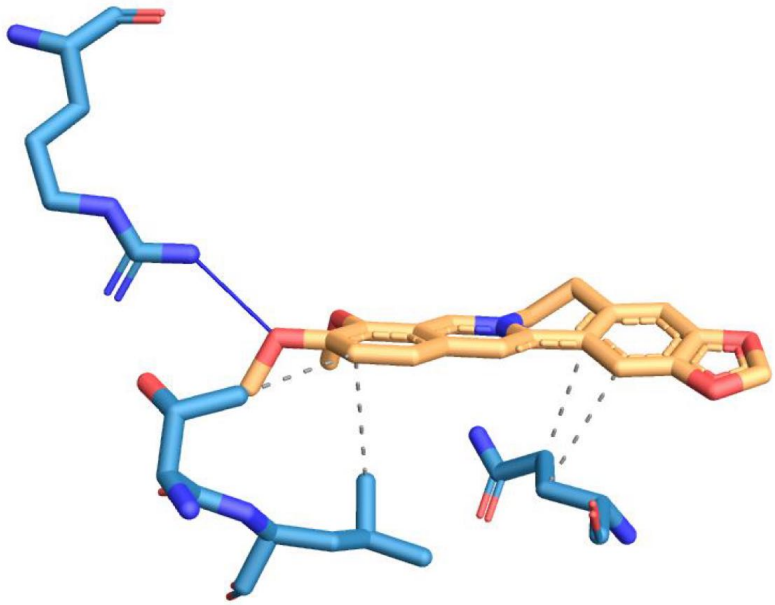
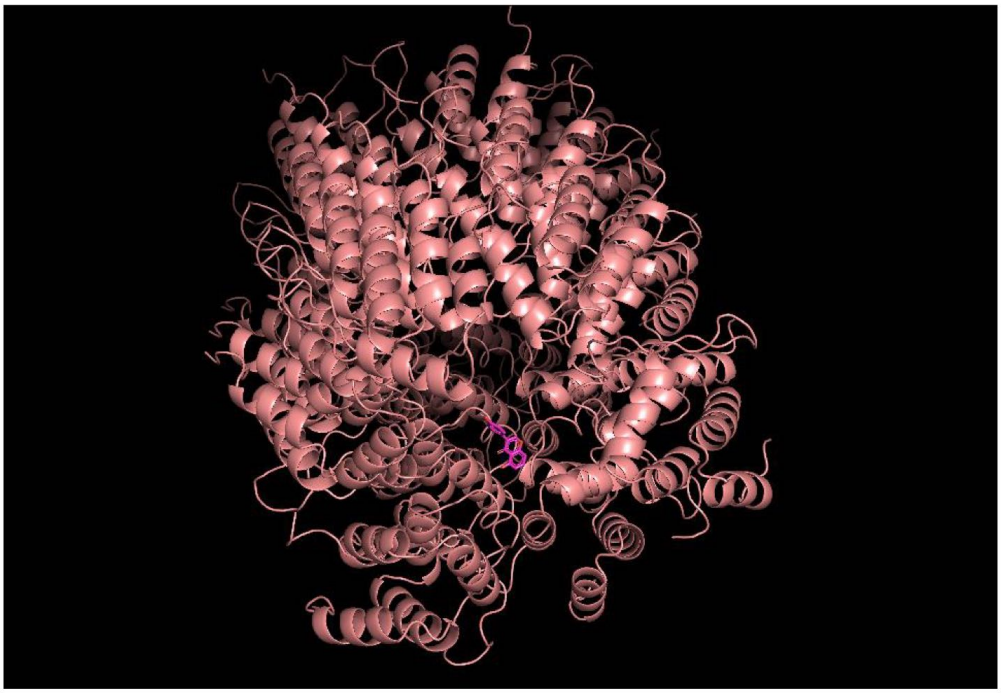


Figure 3. Interaction of Berberine with NLRP3 and its PLIP analysis.



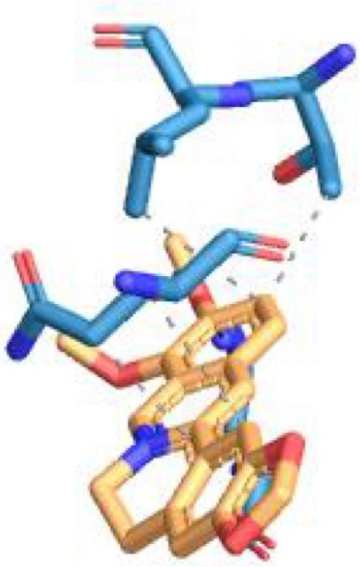
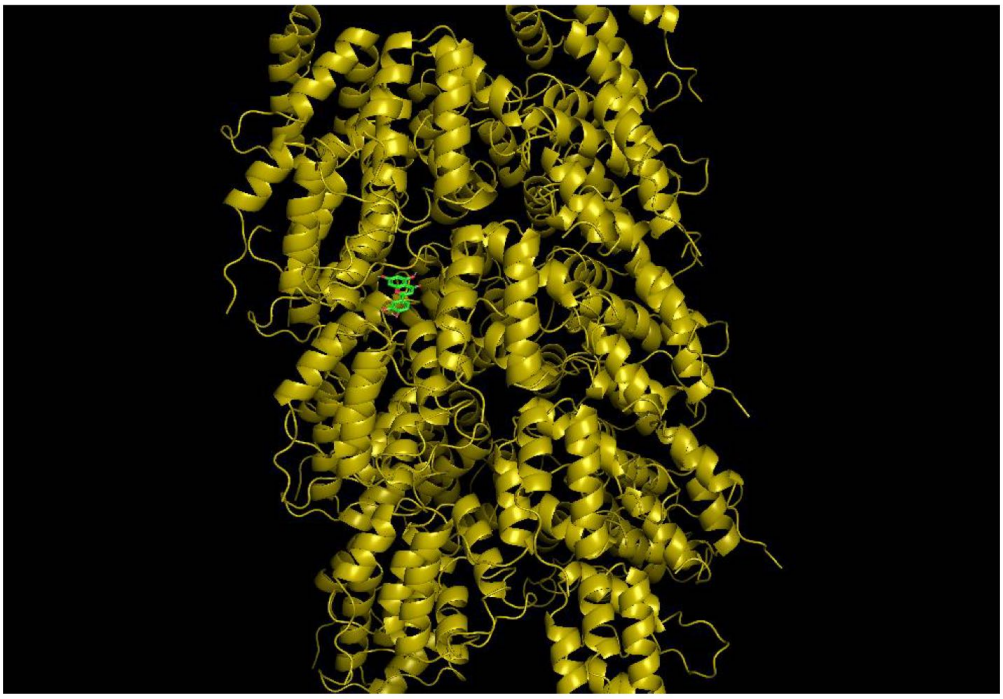


Figure 4. Interaction of Genistein with NLRP3 and its PLIP analysis.



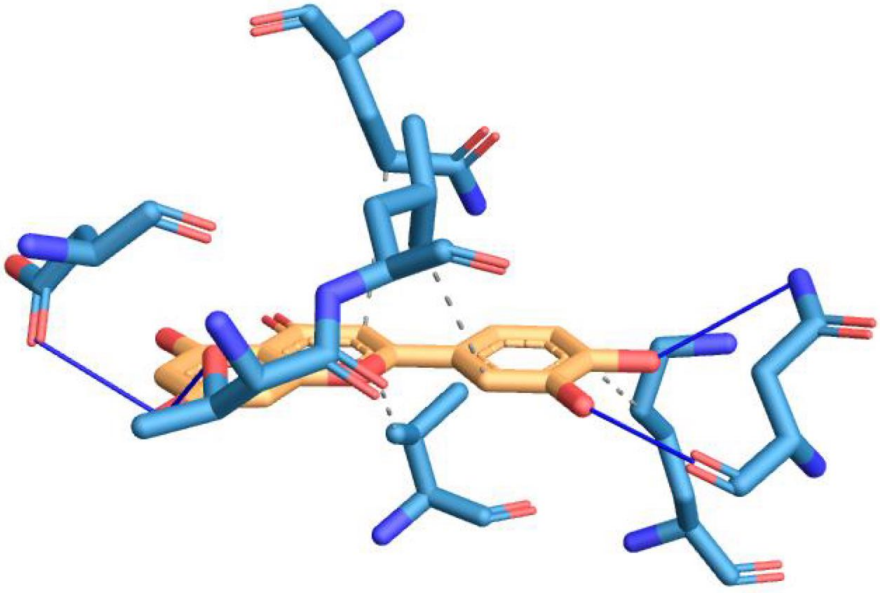


Figure 5. Interaction of Luteolin with NLRP3 and its PLIP analysis.

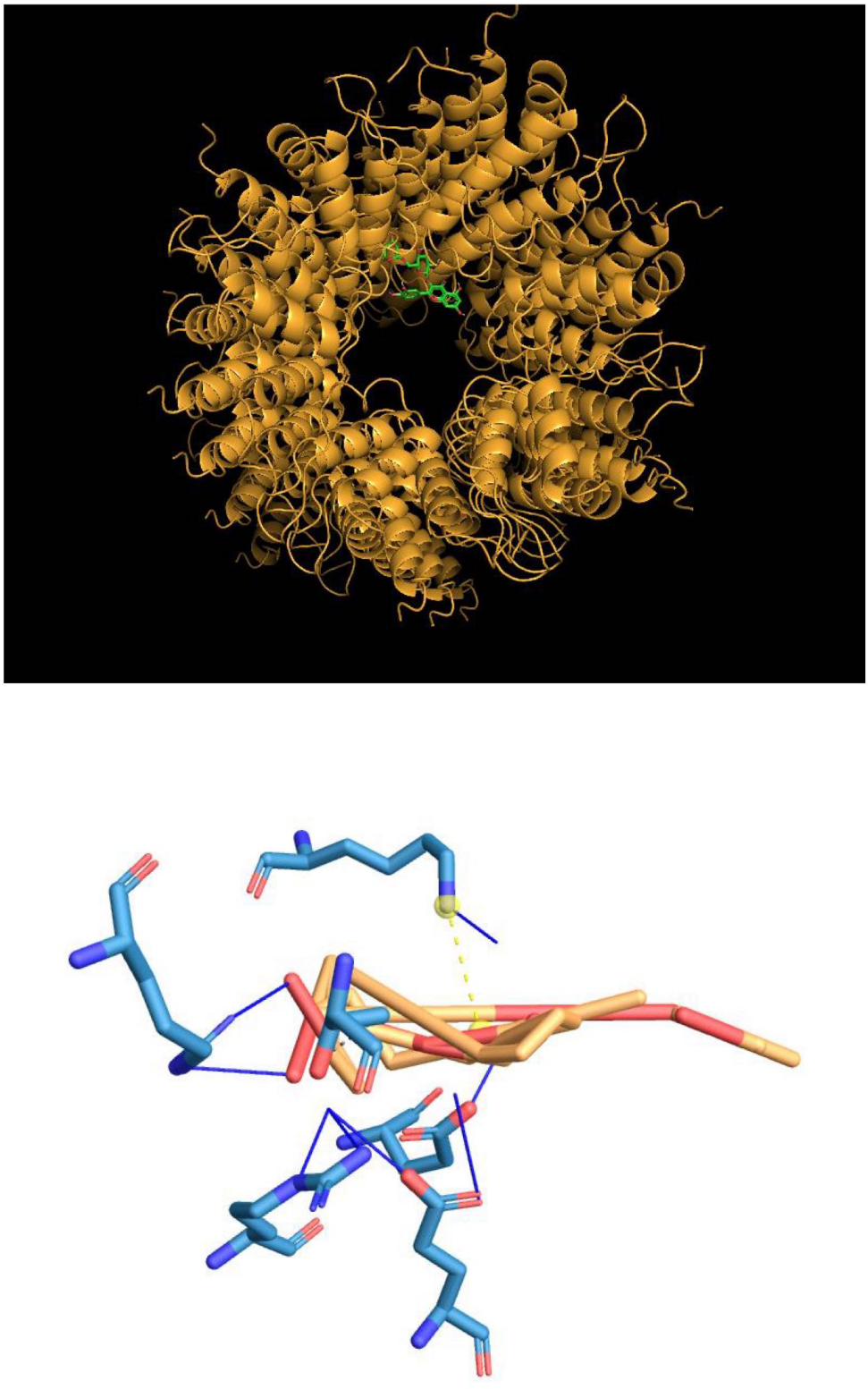


Figure 6. Interaction of Rutin with NLRP3 and its PLIP analysis.

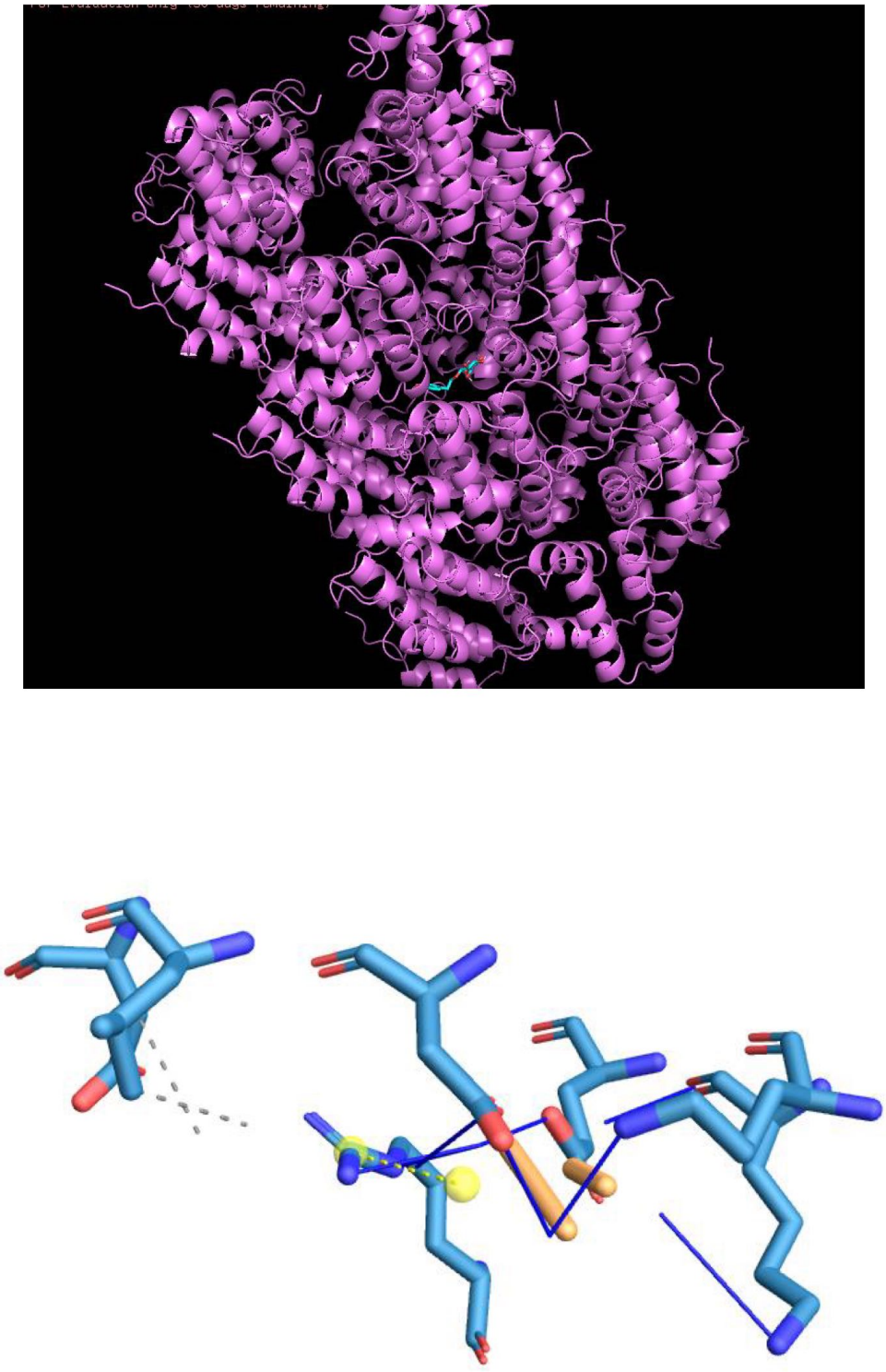


Figure 7. Interaction of and Salidroside with NLRP3 and its PLIP analysis.

CHAPTER 5- OBSERVATIONS

LIGAND	BINDING ENERGY	BIOAVAILABILITY SCORE	LIPINSKI	GI ABSORPTION
1 Andrographolide,	-7.7	0.55	Yes, 0 violation	High
2 Baicalein	-7.9	0.55	Yes, 0 violation	High
3 Berberine	-7.9	0.55	Yes, 0 violation	High
4 Genistein,	-7.5	0.55	Yes, 0 violation	High
5 Luteolin	-7.7	0.55	Yes, 0 violation	High
6 Rutin	-8.6	0.17	No, 3 violations	Low
7 Salidroside	-7.8	0.55	Yes, 0 violation	High

Table 1- Binding energy between Protien(NLRP3) and all the ligands along with ADME Assay analysis of Ligands.

CHAPTER 6- CONCLUSION AND FUTURE PROSPECTS

Based on recent studies, the treatment approach for diabetic cardiomyopathy (DCM) that targets the NLRP3 inflammasome has substantial potential. In conclusion, an essential mechanism inclusively participate in the inflammatory response and the progression of DCM is the NLRP3 inflammasome. By preventing or altering the activation of this protein, it is possible to suppress oxidative stress, inflammation as well as fibrosis, which ultimately enhance the structural together with functional integrity of the heart in persons with DCM.

Several preclinical investigations have shown how well NLRP3 inflammasome inhibitors work to treat diseases connected to DCM. These inhibitors help reduce heart fibrosis and inflammation by reducing the productivity of pro-inflammatory cytokines like IL-1 and IL-18. Degeneration of the NLRP3 inflammasome had also been shown in order to support cardiac remodelling, preserve cardiac contractility, and enhance cardiac function in animal models of DCM. These findings show the efficacy of targeting the NLRP3 inflammasome as a DCM curative strategy.

Aiming towards NLRP3 inflammasome in DCM therapy holds promise for the future. The specific processes underpinning NLRP3 inflammasome activation in DCM and its interaction with other pathways implicated in the development of the disease must be clarified through additional research. The creation of more specialised and potent inhibitors of the NLRP3 inflammasome will be made possible by an understanding of these intricate relationships.

Additionally, to validate the preclinical results and evaluate the safety and effectiveness of NLRP3 inflammasome inhibitors in people subjects with DCM, clinical trials are required. These investigations will contribute significantly to the creation of cutting-edge treatment plans for DCM patients by offering insightful information on the therapeutic potential of NLRP3 inflammasome targeting in a clinical setting.

Additionally, combination therapy incorporating NLRP3 inflammasome inhibitors and other interventions focusing on various DCM pathology-related issues, such as metabolic anomalies,

oxidative stress, and cardiac remodelling, may provide synergistic effects and better therapeutic results. A complete strategy for treating DCM can be achieved by simultaneously focusing on several routes, addressing the disease's complex character.

In summary, NLRP3 inflammasome targeting is a viable treatment strategy for DCM. To improve cardiac structure and function, NLRP3 inflammasome activation can be inhibited. This will also minimise cardiac fibrosis & oxidative stress. To fully explore substancial possibility of NLRP3 inflammasome targeting in DCM therapy and transfer these findings into efficient treatments for people with DCM, more research, including clinical trials, is necessary.

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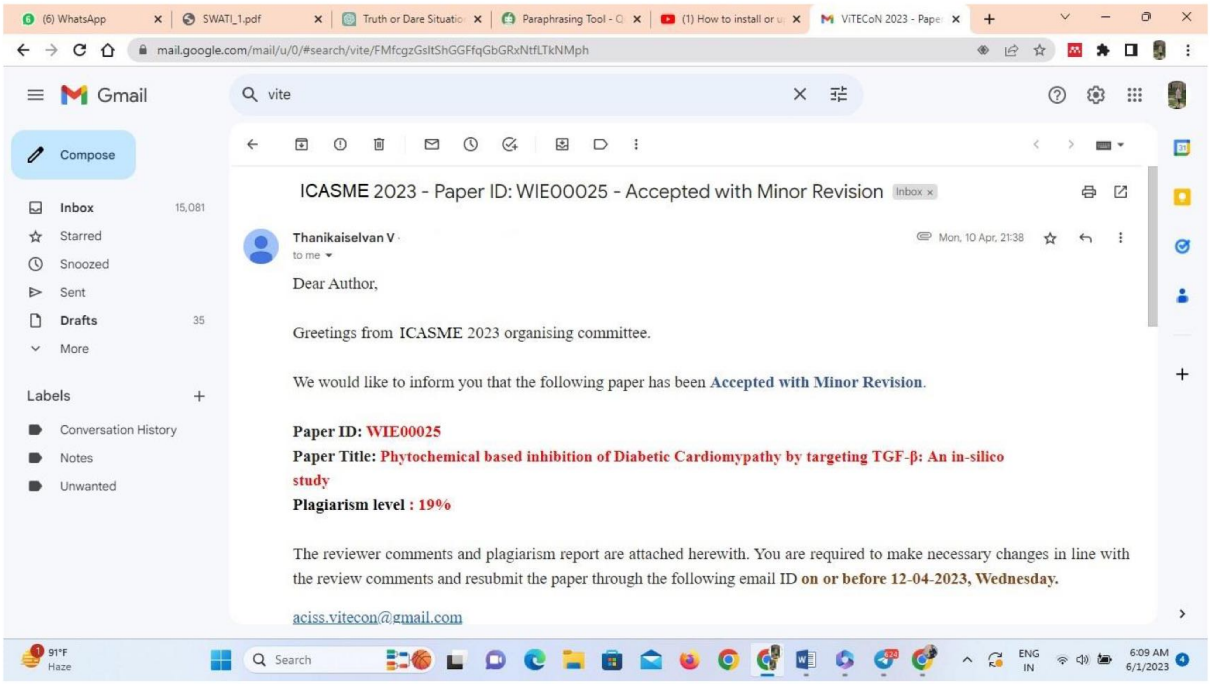

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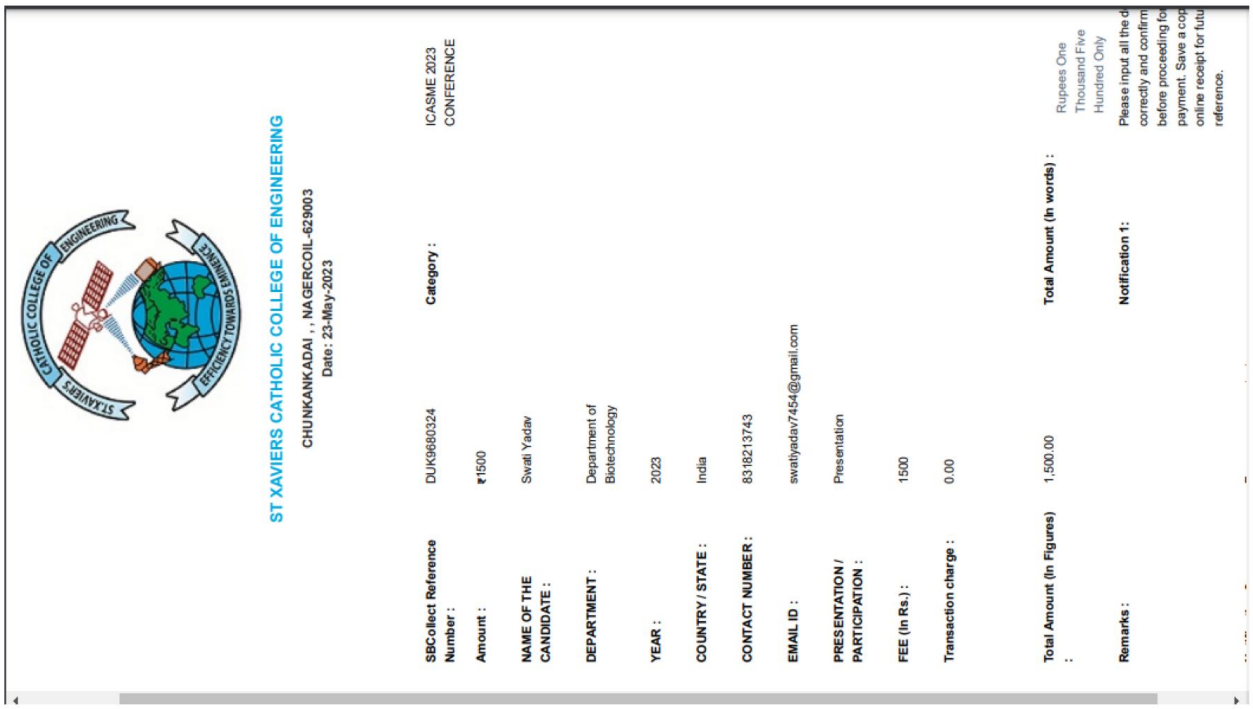

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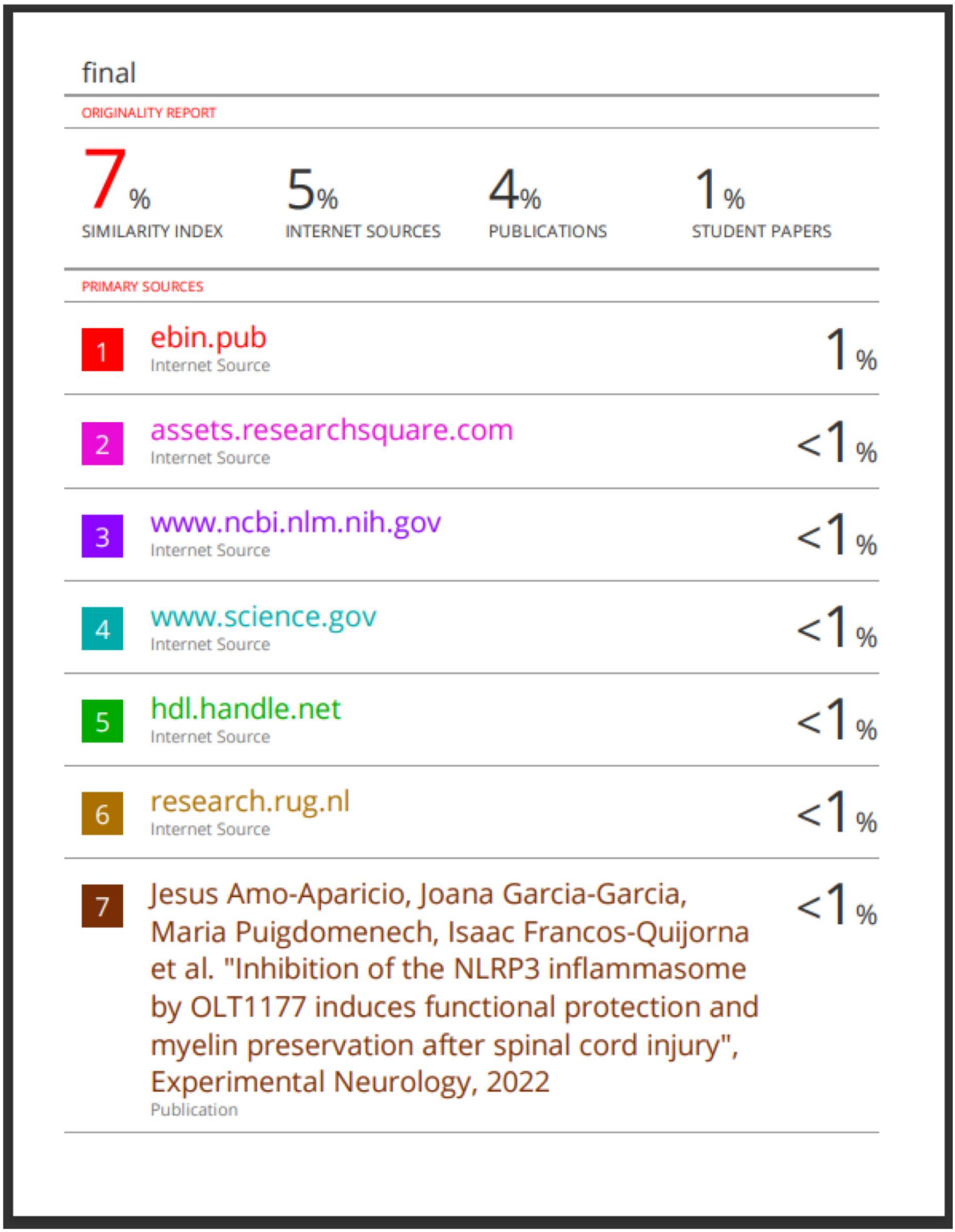

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