

**INTEGRATIVE NETWORK
PHARMACOLOGY AND MOLECULAR DOCKING ANALYSIS
TO ELUCIDATE THE MULTI-TARGET MECHANISM OF KOUMINE IN
NEUROPATHIC PAIN**

Thesis submitted in partial Fulfilment of the Requirements

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BY**

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I, Wakeeta Sharma, 24/IBT/14, Student of MTech Industrial Biotechnology hereby declare that the project work entitled as “**Integrative Network Pharmacology and Molecular Docking Analysis to elucidate the Multi-Target Mechanism of Koumine in Neuropathic Pain**” submitted to the Department of Biotechnology, Delhi Technological University, Delhi, by me for the award of the degree of Master of Technology, is an original work under the guidance and supervision of my supervisor **Prof .Praveer Kumar**. I also certify that this material has not been presented previously for the purposes of the award of any other degree or diploma at any university or learned institution.

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Certified that **Wakeeta Sharma(24/IBT/14)** has carried out their research work presented in the thesis entitled **“Integrative Network Pharmacology and Molecular Docking Analysis To Elucidate the Multi-Target Mechanism Of Koumine In Neuropathic Pain”** for the award of **Master of Technology** from Department of Biotechnology, Delhi Technological University, Delhi, under my supervision. The thesis embodies result of original work and the studies are carried out by the student herself and the contents of the thesis do not form the basis for the award of any other degree to the candidate or to anybody else from this or any other University/ Institution.

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**INTEGRATIVE NETWORK PHARMACOLOGY AND MOLECULAR DOCKING
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WAKEETA SHARMA**

ABSTRACT

The neuropathic pain (NP), occurring in 7-10% of the world population, originates from the somatosensory nervous system damage and is poorly treated by conventional drugs with less than 50% response rate. Although its chemical mechanism is unclear, koumine, an indole alkaloid from *Gelsemium elegans* Benth., showed promise in treating NP. In order to comprehend the multi-target mechanism of koumine in treating NP, this study integrated network pharmacology and molecular docking approaches. Ten overlapping targets between koumine and the target gene list extracted from Gene Cards and OMIM database were found as shared targets for koumine in treating NP. By analysing PPI network with STRING and Cyto-scape tools, four hub genes (KIT, EGFR, SLC6A4, and ABCB1) were selected by Maximal Clique Centrality (MCC). GO analysis indicated that PI3K/PKB signalling pathway, MAPK cascade activation, and serotonergic signalling pathway were important biological processes, with plasma membrane localization and protein tyrosine kinase activity as key cell component and molecular function features. Ten KEGG pathways (adj. $p < 0.05$) were obtained with PI3K-Akt signalling ($-\log_{10}p \approx 1.70$) ranked first among all of them. Binding interaction studies showed that the molecule koumine has favourable binding affinity with all the hub proteins, where the strongest affinity is found to be SLC6A4 (-9.011 kcal/mol). ADMET analysis predicted high GI absorption (98.06%), BBB permeability, and drug-likeness based on the rule of five of Lipinski. This study clearly defines the multi-target approach of the drug koumine towards NP, validating its future experimentation.

Keywords: Koumine, Neuropathic Pain, Network Pharmacology, Molecular Docking, PI3K-Akt Signalling, KEGG Enrichment, Gene Ontology, ADMET, Multi-target, *Gelsemium elegans*.

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LIST OF ABRREVIATIONS

Abbreviation	Full Form
ABCB1	ATP-Binding Cassette Sub-Family B Member 1
ADME	Absorption, Distribution, Metabolism, and Excretion
AMES	Ames Mutagenicity Test
ATP	Adenosine Triphosphate
BBB	Blood-Brain Barrier
BP	Biological Process
CD4+	Cluster of Differentiation 4
CC	Cellular Component
EGFR	Epidermal Growth Factor Receptor
GEB	Gelsemium elegans Benth.
GI	Gastrointestinal
GO	Gene Ontology
HTR2A	5-Hydroxytryptamine Receptor 2A
MCC	Maximal Clique Centrality

CHAPTER -1

INTRODUCTION

The cause of neuropathic pain (NP) is associated with problems in the nervous system. It can arise through mechanisms that make people hypersensitive both externally and internally. Millions of people around the world suffer from neuropathic pain due to neurological problems or damage. Therefore, it is essential to devise new approaches and agents to manage this condition. Researchers have found that neuropathic pain affects 7-8% of the population.

There are two types of neuropathic pain: Peripheral neuropathy (PN) results from problems in peripheral nerves, including sensory and motor nerves, their axons, and myelin sheaths.

The origin of neuropathic pain (NP) lies within the dysfunction in the nervous system. This condition may arise from sensitizing mechanisms both internally and externally. Millions of people throughout the world suffer from neuropathic pain because of damage or impairment to their nervous system. Therefore, there is an absolute necessity to devise novel treatments for neuropathic pain and find suitable drugs.

According to specialists, 7-8 percent of the general public suffer from neuropathic pain, and this percentage will only rise in the coming days. Neuropathic pain is more difficult to treat than nociceptive pain. Neuropathic pain requires a unique mechanism compared to other kinds of pains.

GABA receptor dysfunction, atypical expression pattern of NKCC1 and KCC2, and reduced expression of GAD and GABA transporters in neuropathic pain.

The use of GABAergic agonists could be helpful in the treatment of neuropathic pain induced by nociceptive stimuli. In addition to GABA receptors, some of the other receptors that could serve as targets for neuropathic pain include pro-inflammatory mediators (TNF- α , interleukin 1, and interleukin 6), endocannabinoids (CB1 and CB2), nitric oxide, serotonin, adrenergic, purinergic, and glutamate receptors.[1]

Studies have estimated that 1–17.9% of the general population experience neuropathic pain (NPP), which is categorized under the chronic pain. Neuropathic pain (NPP) is described as dysfunction or damage of the somatosensory system that occurs due to several reasons. It is estimated that the prevalence of neuropathic pain lies within 6.9% to 10%. [2]

GABAergic agonists could help relieve the pain caused due to the nociceptive stimuli associated with neuropathic pain. Other than GABA receptors, neuropathic pain may be treated with endocannabinoids (CB1 and CB2), pro-inflammatory mediators (TNF- α , interleukin-1 and -6), nitric oxide, serotonin, adrenergic, purinergic, and glutamate receptors and their corresponding transporters. *Gelsemium elegans* Benth., a medicinal herb, contains the alkaloid koumine.

Koumine exhibits both analgesic and anti-inflammatory effect; however, the exact mechanism behind it is not known yet. Even though there is an extensive research conducted on the topic. In this research, it was found that the chemical compound Koumine, extracted from *Gelsemium elegans*, is very effective in causing anti-inflammation and analgesic responses. This particular research highlights that the reason for this effect is the inhibition of microglia and astrocyte activity, thus preventing the 'inflammatory storm' post-nervous tissue damage. Also, Koumine can work as a modulator of TSPO.

It was found that administration of koumine in either one dose or repeated doses provides substantial relief from neuropathic pain resulting from nerve damage.[3]

Neuropathic pain is a condition that does not have any cure due to its many causes and complexities in its mechanism of action. The side effects that arise from the current treatment drugs are such that they make their use impossible. Neuropathic pain is considered a category of diseases and not one singular condition, and it exhibits various symptoms. There may be various reasons for the occurrence of neuropathic pain. However, it is critical that neuropathic pain is correctly diagnosed; however, up to date, there is no universally accepted technique for diagnosing it.[4]

Generally, antidepressants or antiepileptics are usually used as the first choice in the treatment; whereas lidocaine plaster, tramadol plus high-concentration capsaicin patches can be used as second options.[5]

Botulinum Toxin A and potent opioids are regarded as the third choice in treating the disease. Unluckily, only 40 to 60 percent of the patients suffering from NPP show any response to the conventional medications currently on the market, and also, such medications are prone to side effects such as anticholinergic side effects, orthostatic hypotension, nausea, and sedation. Therefore, when it comes to the treatment of NPP, it is essential to pay close attention to more potent painkillers with new mechanisms and fewer adverse effects. In phytochemical studies,

the most active components of GEB were reported to be alkaloids which includes koumine, gelsemine, gelsenicine, and gelsevirine are examples of indole alkaloids.

Further investigation was made about GEB and its alkaloid because of their effective pharmacological properties in managing NPP. Both inflammatory pain and neuropathic pain are normally regarded as the most widespread types of chronic pain. At present, the major drugs used for treating chronic pain are NSAIDs.[6]

Given the unique structural composition that makes it distinct from all other pain medications available, koumine is an excellent alkaloid component. Discovered in 1931, its structure was elucidated only in 1981. It should be noted that koumine is a crucial alkaloid compound, being the chief one in *Gelsemium elegans*. Its molecular formula is $C_{20}H_{22}N_2O$, while the molecular weight is 306.4 g/mol. Koumine has a molecular weight of 306.40 g/mol and a formula $C_{20}H_{22}N_2O$. One of the commonest chemicals of *G. elegans* Benth. Unlike other alkaloids, koumine has very low toxicity .[7]

This is why there has been an increased interest in the examination of the potential application of koumine for medical purposes.

Koumine belongs to the group of indole alkaloids and is totally different from all other groups of analgesics available today in terms of its chemical composition . In addition, Koumine does not seem to have any side effects associated with other analgesics available at present. The primary active compound of *G. Elegans* is koumine, which falls under the class of alkaloids. Koumine has a great potential for being used as a painkiller and anti-anxiety drug. In the earlier study, we have demonstrated that koumine has great anti-inflammatory action through blocking the MAPK/ERK, p38, and NF- κ B pathways, resulting in reduced inflammation mediator and cytokine production.[8]

In contrast, while it has no impact on any other autonomic nerve or physiological functions, low doses of koumine have been found to lower anxiety. The mechanism behind this can be associated with its agonistic activity at glycine receptors in addition to increasing the concentrations of neurosteroids such as pregnenolone and allopregnanolone in the hippocampus [9].

The compound, Koumine, inhibits CD4⁺ T cell proliferation and reduces their immune response in vitro . Various dosages of koumine are effective in suppressing the cytokines produced by Th1 cells and in significantly lowering the levels of IL-2 and IFN- γ ; indeed, at the

concentration of 100 $\mu\text{g/mL}$, koumine increases the level of IL-10 without increasing IL-4 levels in Th2 cells .Additionally, the process of complement-mediated hemolysis is suppressed by higher levels of koumine . The anti-psoriasis property of koumine has been proven due to the ability of koumine to inhibit epithelial cell hyperproliferation and promote keratinization of the epidermal layer to form a granular layer .[10]

Even though there has been a lot of study on koumine 's regulation of neuropathic pain, the molecular mechanism behind this process remains unknown to this day. Even though network pharmacology helps us predict the ligands and molecular pathways that interact with one another, experimental studies do not always support ligand-protein connections. It is through molecular docking techniques that computer simulations help in the study of how proteins and tiny molecules interact .To close this knowledge gap and shed light on possible mechanisms for drug treatment. Koumine was therefore docked on proteins associated with neuropathic pain.[11]

Objective : To investigate the therapeutic potential and molecular mechanism of Koumine against neuropathic pain using network pharmacology, molecular docking.

CHAPTER – 2

LITERATURE REVIEW

2.1 Introduction to Neuropathic Pain

Complex persistent pain known as neuropathic pain is brought on by a malfunction of the somatosensory nervous system. Unlike nociceptive pain, neuropathic pain includes abnormal neuronal signals even in the absence of stimuli. Neuropathic pain includes such symptoms as burning, tingling, hyperalgesia, and allodynia. It affects about 6-10% of the world's population, imposing a significant burden on the healthcare system [12], [13].

Generally, neuropathic pain can be classified into two types, that is, peripheral and central. Although peripheral neuropathic pain results from conditions such as diabetes mellitus, chemotherapy-induced neuropathic pain, and peripheral nerve damage, this kind of pain is associated with the failure of peripheral nerves. Contrarily, central neuropathic pain results from central nervous system pathology or malfunction such such as spinal cord injury, multiple sclerosis, and stroke [13], [14].

There are various underlying causes of neuropathic pain.

Several pathophysiological mechanisms have been attributed to neuropathic pain, one of which being neuroinflammation. Neuroinflammation involves the activation of immune cells, for instance, astrocytes and microglia and damage to nerves, resulting in the release of pro-inflammatory substances like chemokines, interleukins, and TNF- α . Inflammatory factors cause sensitization and excitation of neurons; hence, increasing pain perception [15], [16].

An additional mediator for neuropathic pain is the imbalance between the body's antioxidants and reactive oxygen species (ROS) which is known as oxidative stress.

An excess of ROS can result in damage to proteins, lipids, and deoxyribonucleic acid (DNA), which in turn will cause dysfunction in the neurons. Oxidative stress may lead to the development of inflammatory states; hence, there will be more pain sensitivity. Another mediator in neuropathic pain is central sensitization, where an increased sensitivity of neurons within the CNS leads to neuropathy. Nociceptor pathway activity results in the production of signals that increase pain sensitivity regardless of whether or not the stimulus is painful.

Various signalling pathways, like PI3K-Akt and MAPK, help in facilitating central sensitization [17]. Moreover, signal transduction pathways involved in inflammation and neurotransmission include protein kinases like EGFR and KIT [18].

2.2 Current Treatments & Limitations

As a result of having an elaborate cause, and being refractory to conventional therapies, NP is still a challenging clinical disease. Currently, antidepressants, anticonvulsants, opioids, and topical drugs are among the common pharmacological therapies for neuropathic pain. Tricyclics like amitriptyline and serotonin norepinephrine reuptake inhibitors (SNRIs) like duloxetine, both of which act on descending inhibitory systems by increasing serotonin and norepinephrine, are commonly administered [19]. Anticonvulsants including gabapentin and pregabalin affect voltage-gated calcium channels to decrease neuronal hyperactivity [20].

Despite the availability of these drugs, fewer than 50% of patients have significant relief from their pains [13].

Opioids, including morphine, although considered to be second-line and sometimes third-line medications, cannot be used indefinitely since there is always the risk of tolerance and dependency. Topical agents, such as lidocaine and capsaicin patches, can help alleviate pain temporarily but may not work on extensive neuropathic pain. The complex characteristics of neuropathic pain represent one of the main reasons for the lack of effectiveness of currently existing medications in managing this condition. Various types of disorders, such as dysfunctional ion channels, oxidative stress, neuroinflammation, and central sensitization, may cause the emergence of neuropathic pain. Regrettably, many of the currently available medications address only one aspect of the disease's mechanism, which makes it impossible to manage its complex aspects. The inconsistency in the reaction to drugs used in treatment is another challenge in the management of neuropathic pain. This fact shows how important it is to have medications able to act on multiple targets at once. Network pharmacology can be regarded as an innovative drug development approach that can help to design drugs with multiple targets.[21]

From this perspective, the possibility of using natural products such as koumine, which could synergistically affect multiple biological pathways, to treat neuropathic pain is promising.

2.3 Natural Compounds in Neuropathic Pain

In view of the fast emerging trends in drug discovery and development, the use of natural substances as a source of medication has experienced a renewal in interest, with phytochemicals derived from medicinal plants emerging as potential therapies for treating

neuropathic pain (NP). These substances offer promise as treatment modalities for complicated NP by possessing a distinct variety of pharmacological characteristics, including neuroprotective, anti-inflammatory, and antioxidant effects [22].

Several molecular mechanisms implicated in the development and maintenance of neuropathic pain (NP) have been shown to be modulated by phytochemicals. Compared with conventional Western medicines which are mostly singular target effectors, plant-derived chemicals can affect a variety of important biological processes including neuroinflammation, oxidative stress, activities of various ion channels, and the synaptic transmission of key neurotransmitters. This property is particularly relevant for NP which is characterized by a diversity of underlying pathological processes, simultaneously interacting to define the clinical manifestation of disease.[23]

Phytochemicals have gained increasing attention as novel therapeutic approaches to treat neuropathic pain. Among them, alkaloids have shown promising analgesic and anti-inflammatory effects. The plant alkaloid berberine has anti-inflammatory and antioxidant effects by lowering oxidative stress and inhibiting the synthesis of pro-inflammatory cytokines. In neuropathic pain models in animals matrine attenuated pain behaviours through effects on inflammatory signalling and reduced neuronal excitability. Sinomenine, isolated from Sinosamin, had analgesic effects by suppressing production of pro-inflammatory cytokines and preventing microglial activation.[24]

Besides the alkaloids, other classes of phytochemicals like flavonoids and terpenoids are known to provide relief from neuropathic pain. The strong antioxidants like flavonoids (e.g. quercetin) counteract oxidative stress and consequent neuronal damage. [25] Many terpenoids, such as curcumin, have been reported to offer relief from pain hypersensitivity through inhibition of inflammation, through interference with NF- κ B and other such signalling pathways. Natural products generally have better safety and lower toxicity than synthetic drugs. Additionally, some natural chemicals have the potential to be developed as multi-target drugs. This is especially relevant to treating complicated disorders such as neuropathic pain from a new perspective of network pharmacology based on multi-Components, multi-Targets and multi-Effects .Despite vast potential as therapy, the phytochemicals are constrained by problems of poor pharmacokinetics and bioavailability, particularly a paucity of extensive clinical trials. However, studies on drug delivery and structural modification of lead molecules are increasingly likely to overcome these constraints and translate active phytochemicals into

effective therapy.[26] Biologically active natural compounds, studied as potential anti neuropathic pain drugs, offer promising leads for future development. Especially in the treatment of complex multifactorial disease such as NP, their diverse actions and multi-target strategies may have advantages.

2.4 Introduction to Koumine

Gelsemium elegans, a native plant in many Southeast Asian countries, is the primary source of the naturally-occurring indole alkaloid koumine. While *Gelsemium elegans* is known to be toxic, the many pharmacological properties have been well-explored especially for its neuroprotective and analgesic effects .[27]

Koumine is a broad spectrum pharmacological agent, exhibiting neuroprotective, analgesic, anxiolytic, and anti-inflammatory actions. Its potential for pain relief is supported by the compound's influence on activity of the central nervous system, which does not produce the major side effects typical of opioid-like analgesics . [28]Koumine also has antioxidant properties, thus providing protective action against oxidative stress-induced diseases and brain damage .[29]

The processes via which koumine reduces neuropathic pain are complex and multifaceted. One major process through which koumine induces its effects is by interacting with the γ -aminobutyric acid (GABA) system to increase GABAergic neurotransmission, which decreases neuronal excitability and prevents pain signalling.

Koumine has been found to have both anti-inflammatory and central nervous system active effects. By reducing neuroinflammation, a major cause of neuropathic pain, it prevents the production of pro-inflammatory cytokines, including TNF- α and interleukins .[30]

By controlling microglia activity, koumine can also have an impact on the central nervous system. Koumine inhibits microglia activation and related neurotransmission pathways which leads to reduced central sensitization and pain hypersensitivity. Activated microglia secrete a variety of inflammatory factors to increase pain sensitivity.

Based on the multi-target effects of Koumine on multiple bio-pathways including the anti-inflammation on neuropathic pain via microglial inhibition and the modulation of GABAergic signalling, Koumine can be used as a promising drug for treating neuropathic pain; and network pharmacology approach can be applied to understand its mechanisms of action; this strategy is in accordance with the new trend of multi-target drug discovery idea.[31]

Although preclinical findings indicate that it is worthwhile to develop as a novel medication for the treatment of neuropathic pain, further research is required to fully understand its pharmacokinetics, safety, and therapeutic effectiveness for clinical usage. It has a wide pharmacological profile.

2.5 Network Pharmacology

Network pharmacology is a new multidisciplinary approach that examines the interactions of drugs, targets and diseases at the level of the system – combining pharmacology, bioinformatics and systems biology. Network pharmacology emphasizes "multi-target–multi-component" strategy which is most suitable for complex disease such as neuropathic pain as opposed to "one drug–one target" concept .[32] The basic premise of network pharmacology is that biological systems function as interconnected networks as opposed to independent pathways. Therapeutic treatments targeting multiple nodes within these networks may yield longer-lasting and more effective results. Over the course of disease, it identifies important targets, signalling pathways and molecular interactions through which a medication produces its action .[33] Network pharmacology is becoming increasingly important in drug discovery today, especially for studying natural chemicals and traditional medicines.

The use of TCMSP, Swiss Target Prediction, STRING databases and computer tool of Network pharmacology to predict possible targets and mechanism of action of phytochemicals of herbs which often involve multiple active constituents.[34]

Network pharmacology is also widely used to find and evaluate targets, as well as to anticipate possible active molecules and mechanisms of action.

The reconstruction of PPI networks and the calculation of topological features allow for the identification of crucial hub genes and their involvement in disease processes. Additionally, the identified hub targets could serve as potential therapeutic options and be used for further experimental validation .[35]

Route enrichment analysis to identify important biological pathways including inflammation, oxidative stress and neurotransmitter related pathways involved in specific disease conditions using the tools of network pharmacology. Since several different overlapping pathways are believed to support the pathophysiology of neuropathic pain, finding a comprehensive "road map" to the variety of drug targets and potential bioactive compounds that modulate them is highly relevant to the field.[36]

In addition, the approaches of the network pharmacology have been applied to investigate how bioactive substances work like koumine. Moreover, the approach of developing multi-target medicines is supported by the ability to integrate chemical, genomic and pharmacological data to predict the simultaneous action on multiple targets and pathways of a given drug.[37]

Network pharmacology is a novel approach to thinking about drug-disease interactions in a comprehensive and organized way which represent a paradigm shift in the process of finding new drugs. For the purpose of treating multifactorial diseases like neuropathic pain, network pharmacology may help to understand the effects of multi-target approach.[37], [38]

2.6. Molecular Docking

A computational method is called molecular docking to predict the binding of a ligand (a drug, a bioactive compound) to target a molecular level of a protein. According to , it helps in determining the orientation, affinity, and stability/strength of the interaction of a ligand in a preferred binding inside the active site of a protein.[39] Owing to its ability to rapidly screen numerous compounds against specific biological targets, this strategy is widely utilized in drug discovery. Molecular docking eases the identification of possible lead drug candidates that have a high affinity and specificity for disease-causing target proteins by calculating binding energies and interaction patterns .[40]

Molecular docking is relatively more affordable than experimental procedures, and this is one of its most significant benefits. Molecular docking is a vital method in present-day pharmacological studies because it significantly reduces the necessity for prolonged in vivo and in vitro experiments in the preliminary stages of drug discovery .[39]

Molecular docking is also required to confirm the targets identified through network pharmacology approaches. To confirm the capability of potential compound-target bindings, docking studies are conducted after the identification of potential binding sites between drugs and target proteins using computational methods .[41]This provides structural confirmation of the biological significance of the selected targets.

Moreover, by recognizing crucial amino acids responsible for ligand interaction and their binding modes and in the understanding of molecular systems, including hydrogen bonding, hydrophobic interactions, and electrostatic forces.

In general, molecular docking is a crucial tool for confirming drug-target interactions and promoting the creation of multi-target treatments, especially in complicated illnesses like neuropathic pain.

2.7. Research Gaps

Being not effective enough and unable to tackle the multifactorial nature of NP, the existing methods of treatment prove inefficient due to remarkable advances that have been made in understanding NP and its management. The current pharmaceutical interventions, although numerous, are directed at specific molecules only, thus being ineffective for providing comprehensive therapy in the case of NP, as they do not cover such factors as central sensitization, oxidative stress, and neuroinflammation. [42]

Although there seems to be promising multivalent effects from natural compounds and phytochemicals, the actual molecular mechanism behind these remains unclear. From preclinical studies, bioactive alkaloids such as koumine have displayed impressive analgesic and anti-inflammatory properties; unfortunately, the understanding of its molecular targets is yet to be uncovered. [43]

Also, although network pharmacology allows for an organized approach to predict drug target interactions, several investigations have not been able to integrate these findings with molecular docking to confirm them, thus leaving room for a missing link between structural validation and predictive computation.

To fill this void and understand the multiple-target effects of koumine in the context of neuropathic pain, this present investigation combines network pharmacology with molecular docking. This approach will help formulate effective multi-target strategies.

CHAPTER 3

METHODOLOGY

3.1 Compound Data

Structure data of the selected bioactive compound such as koumine was acquired from the PubChem repository (<https://pubchem.ncbi.nlm.nih.gov/>) which is a database that is accessible. It consists of chemical substances and their biological activities. The chemical properties including canonical SMILES, molecular formula, and 3D structure of the chemical were obtained after searching for the drug through its common name. These structures were exported in SDF format as they were useful for future computational analysis.

3.2 Prediction of the Target

Expected Protein Objectives for the Chosen Substance. The Swiss Target Prediction website (<http://www.swisstargetprediction.ch/>) and the Traditional Chinese Medicine Systems Pharmacology (TCMSP) database (<https://old.tcmsp-e.com/tcmsp.php>) were used to predict potential protein targets for the chosen drug. Based on the assumption that similar compounds would bind to the same protein targets, the Swiss Target Prediction tool uses both two-dimensional (2D) and three-dimensional (3D) similarity metrics to suggest protein targets. In this case, the species was specified as Homo sapiens, while the canonical SMILES code of the chemical was provided as an input to predict human targets. The obtained protein names were then converted to genes according to the Uni Prot database (<https://www.uniprot.org/>).

3.3 Collection of Disease Targets

The gene targets related to diseases such as neuropathic pain were collected from two databases, namely, OMIM (<https://www.omim.org/>) and Gene Cards (<https://www.genecards.org/>). To retrieve relevant genes for neuropathic pain, the keyword "neuropathic pain" was used. In order to ensure that the retrieved genes have high correlation with neuropathic pain, the genes obtained from the Gene Cards database were further selected based on their scores. The genes that are strongly correlated genetically and clinically with neuropathic pain were identified via OMIM.

3.4 Identification of Common Targets

The commonality between drug targets and disease targets was established in order to identify any potential target areas for treatment by the drug on neuropathic pain. The process of identifying the targets is done using Venn diagrams (for instance, with the aid of Venn diagram websites such as Venny 2.1).

The genes that overlap in their functions are selected as the potential target areas for further study as they are considered common target areas. These targets represent the potential sites for action of the drug on neuropathic pain.

3.5 Building Protein–Protein Interaction (PPI) Networks

In order to determine protein-protein interactions, the proteins identified were added to the STRING database (<https://string-db.org/>). Both direct and indirect links among the proteins are merged through STRING. In order to have reliable protein-protein interactions, an adequate 0.4 is the threshold interaction score which was used and the parameter “species” was set as “Homo sapiens.” Data about the network of protein-protein interactions were exported as TSV files that include nodes (the proteins) and edges (the interactions). After that, the network was entered into Cyto-scape for further analysis and visualization. The Cyto-scape platform provides an environment for visualizing complex networks and analysing their topological properties. In order to visualize the network better, algorithms such as "Prefuse Force Directed" were applied.

3.6 Hub Gene Identification

For the purpose of determining the importance of the PPI network genes, Topological analysis was performed using the Cyto-Hubba plugin in Cyto-scape software. Cyto-Hubba is widely used software to determine node ranks on the basis of various centrality scores. Centrality scores of each node were determined using various approaches such as Degree centrality and Maximal clique Centrality (MCC), and higher scores indicated the importance of the node to maintain biological and network functionality. Hub genes were selected from the best-scoring genes, i.e., 5–10 genes. Top targets selected on the basis of their topological centrality score were subjected to enrichment analysis and molecular docking due to their crucial importance for pharmacological activity.

3.7 KEGG Pathway Gene Ontology-Based Enrichment Analysis (GO)

Gene ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways

enrichment studies were carried out utilizing online resources such as DAVID (<https://david.ncifcrf.gov/>) and Meta-scape (<https://metascape.org/>) in order to investigate biological processes and pathways connected to shared aims.

The common targets were classified into three biological functions through GO enrichment analysis it comprised cellular component (CC), molecular function (MF), and biological process (BP). In order to identify significantly enriched pathways associated with the targets, KEGG pathway analysis was performed based on their significance and association with neuropathic pain, the most highly enhanced KEGG pathways and GO keywords. The findings have been presented in form of bar plots and bubble plots for better understanding.

3.8 Building Networks

The use of Cyto-scape software was used to design network models that would help provide more insights into the multiple target and pathway action mechanisms of the compound under study. Networks included in this analysis were:

3.8.1 Compound Target Network

In this network, we see the relationship between the selected compound and its expected pharmacodynamic targets. The chemical as well as the target proteins are indicated using the node while the edges represent the interaction among these nodes.

3.8.2 Target-Pathway Network

This network helps identify the relationship between the enriched KEGG pathways and critical targets. Genes and pathways were indicated using the node while the edge represents the target of interest in certain biological functions.

3.9 Analysis of ADMET

Using in silico methods, the pharmacokinetic and toxicity characteristics of the chosen drug, Koumine, were assessed. The compound's canonical SMILES was obtained from PubChem.

Swiss ADME (<https://swissadme.ch/>) was used to forecast the drug-likeness, distribution, and absorption characteristics. Additionally, pk CSM (<https://biosig.lab.uq.edu.au/pkcsml/>) was used to evaluate toxicity parameters such as hepatotoxicity, AMES toxicity, and other pharmacokinetic characteristics. The compound's pharmacological potential and safety profile were assessed by analysing the acquired results.

3.9.1 Docking of Molecules

To ensure the compound interaction with the important targets proteins, studies using molecular docking were carried out. To conduct these studies, the protein 3D structures available within the PDB database (<https://www.rcsb.org/>) were downloaded. After this, hydrogen atoms were added, while water molecules were removed. In addition, optimization for docking was conducted. Through energy minimization, the compound structure (ligand) was constructed using the appropriate software. For example, such programs as Auto Dock () and Py Rx() can be used for the process. The protein's binding site was covered by the grid parameters set. scores were calculated using the docking results. They reflected the compound (ligand)-protein binding affinities. Important interactions between the target proteins and the ligand were detected based on the docking results.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Finding Common Genes:

To find overlapping targets, two gene datasets were examined. There were 100 genes in List 1 and 299 genes in List 2. To identify shared genes between the two lists, a Venn diagram was created using Venny 2.1. Ten overlapping genes were identified by this analysis: SIGMAR1, SLC6A4, OPRK1, HTR2A, SCN5A, ABCB1, JAK2, KIT, EGFR, and PRKCG (Figure 1).

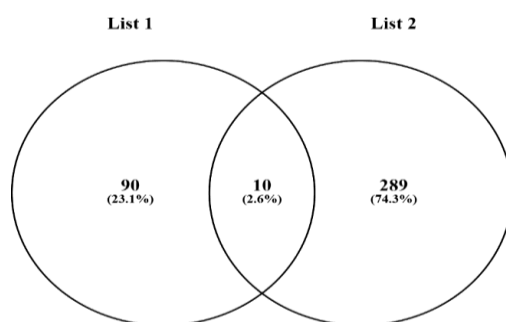


Fig 1: The following figure displays the Venn diagram demonstrating commonality among koumine targets and the associated genes in neuropathic pain. List 1 consists of koumine predicted protein targets (n = 100) and List 2 contains Breast Cancer associated genes (n = 299). The intersection area highlights the 10 common targets (2.6%) that were considered potential core therapeutic targets.

4.2. Protein-Protein Interaction (PPI) Network Analysis:

The 10 common genes were put into the STRING database in order to construct a PPI network. An interaction with great confidence score threshold of 0.7 was used, and Homo sapiens was chosen as the organism. To concentrate on dependable relationships, disconnected nodes were concealed. With six nodes and five edges, the resulting network formed two separate functional clusters: Signalling cluster: KIT, EGFR, and JAK2, suggesting a central hub probably connected to proliferative and inflammatory processes. Serotonergic signalling components are represented by the neurotransmission cluster HTR2A and SLC6A4. Due to the lack of high-

confidence interactions, five genes (SIGMAR1, OPRK1, SCN5A, ABCB1, and PRKCG) were excluded from the network visualisation.

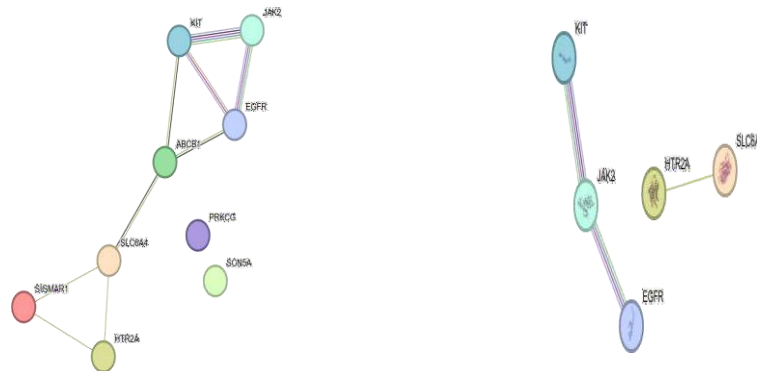


Figure 2: Building the hub gene subnetwork and protein–protein interaction (PPI) network. (A) Cyto-scape software was used to analyse the PPI network of overlapping genes that was produced from the STRING database (score > 0.7). Target proteins are represented by the nodes, while protein interactions are shown by the edges. Interaction evidence types are shown by edge colours.

4.3 Hub Gene Identification:

Seven hub genes were found based on topological characteristics (degree, betweenness, closeness centrality) by using Cyto-scape to analyse the PPI network built from overlapping Koumine targets and neuropathic pain genes. Here, KIT, EGFR, SLC6A4, and ABCB1 were found to be hub genes.

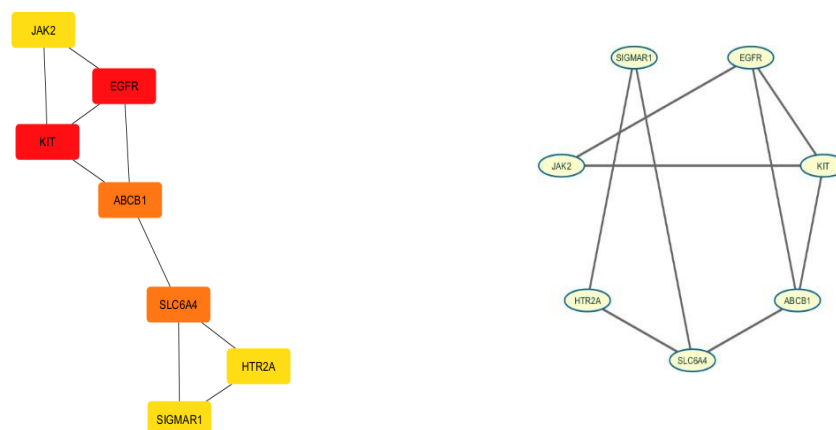


Figure 3 : Protein-Protein Interaction (PPI) networks are displayed by neuropathic pain targets. Hub genes were identified using the Maximal Clique Centrality (MCC) algorithm and the cyto-Hubba plugin. In the colour gradient, lower-ranked nodes are shown as orange and yellow, while the top-ranked hub genes are shown as red.

Table 1: Showing the top four identified hub genes from cyto-hubba on basis of MCC Score

Rank	Node (Gene)	MCC Score (Colour)
1	KIT	Red (Highest)
1	EGFR	Red (Highest)
3	SLC6A4	Orange
4	ABCB1	Orange

4.4 Network Pharmacology:

To systematically elucidate the biological processes via which koumine acts to exert therapeutic effects in neuropathic pain conditions, the common target genes were used for KEGG pathway enrichment analysis. Ten statistically significant pathways were obtained as results of the analysis (adjusted $p < 0.05$). The list included signalling, receptor activity, and metabolic pathways. As the most statistically significant pathway with the highest number of targets (adjusted $p \approx 0.02$; $-\log_{10}p = 1.70$; targets = 3) the PI3K-Akt signalling pathway was recognized. The relevance of this result is highlighted by the fact that PI3K/Akt axis has been extensively studied regarding its role in peripheral and central sensitization, neuroinflammation, and survival of pain-mediating sensory neurons. Another category that stood out from the other terms as statistically significant was the broad category of "Pathways in cancer" (adjusted $p \approx 0.08$; targets = 3) and the more specified one called "Carbon metabolism in cancer" (adjusted $p \approx 0.07$). The latter two pathways indicate mechanistic similarity of signalling processes in cancers and neuroinflammatory disorders, as was previously discussed in literature. As another enriched pathway (adjusted $p \approx 0.065$), the enrichment of the EGFR TKI resistance pathway indicates that EGFR-mediated signalling may be in addition to the PI3K/AKT pathway, the PD-1 checkpoint pathway (adjusted $p \sim 0.06$) was significantly enriched, suggesting an involvement of koumine in neuroimmune interactions in the pain milieu since immune checkpoint ligands were recently shown to be involved in nociception regulation. The Serotonergic synapse pathway (adjusted $p \sim 0.04$) is of special interest since it directly implicates koumine in the regulation of serotonergic neurotransmission – an essential part of descending pain modulation systems. Finally, the enrichment in Gap junction pathway (adjusted $p \sim 0.055$) suggests another possible mechanism of action associated with regulation of glial cell communication, one of the key mechanisms in charge,

of the development of central sensitization during chronic neuropathic pain. Taken together, these data suggest that koumine acts via several pathways with PI3K/AKT signalling and neuroimmune modulation being the main modes of action.

Rank	Pathway	$-\log_{10}(p)$	Adj. p-value	Targets
1	PI3K-Akt signalling pathway	~ 1.70	~ 0.02	3
2	Pathways in cancer	~ 1.42	~ 0.08	3
3	Carbon metabolism in cancer	~ 1.38	~ 0.07	2
4	EGFR TKI resistance	~ 1.30	~ 0.065	2
5	PD-1 checkpoint pathway	~ 1.27	~ 0.06	2
6	Gap junction	~ 1.27	~ 0.055	2
7	Serotonergic synapse	~ 1.15	~ 0.04	2
8	Breast cancer	~ 1.05	~ 0.03	2
9	Phospholipase D signalling	~ 1.05	~ 0.03	2
10	Gastric cancer	~ 1.05	~ 0.03	2

Table 2. KEGG Pathway Enrichment Analysis of Shared Targets for Koumine and Neuropathic Pain. The top 10 KEGG pathways found to be significantly enriched through functional enrichment analysis on 10 shared targets.

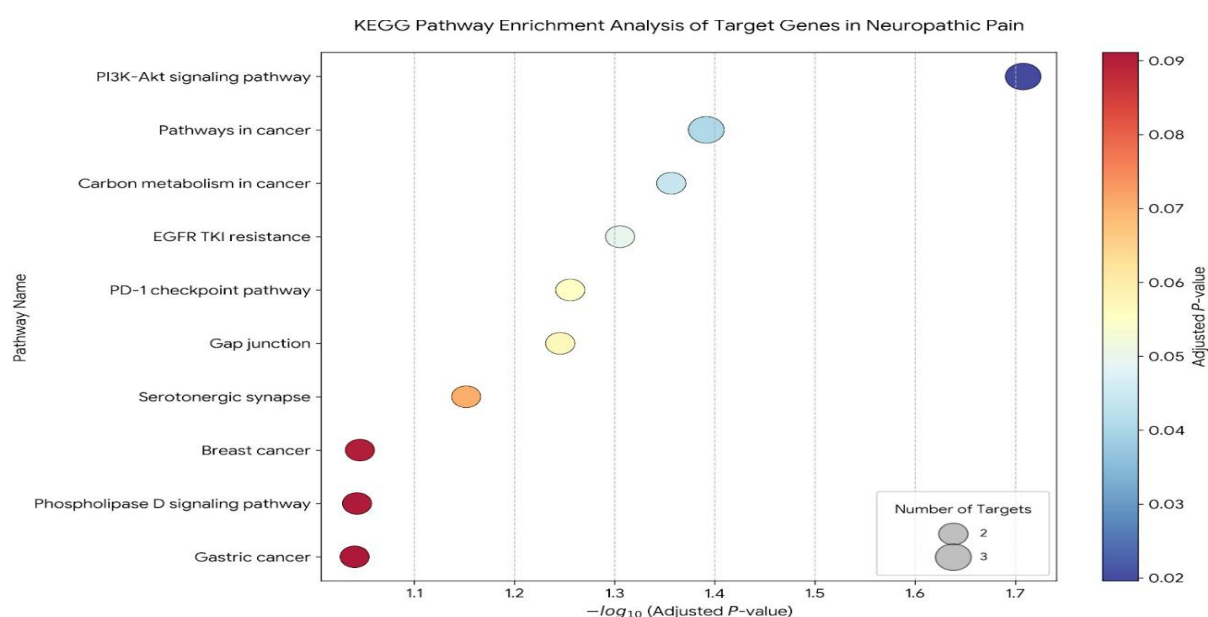


Fig 4: Target gene enrichment analysis using the KEGG pathway in neuropathic pain.

4.5 GO Analysis of Functional Enrichment:

To clarify the biological mechanisms mediated by the identified koumine target genes in neuropathic pain, GO functional enrichment analysis was conducted using the BP, CC, and MF categories (Figure X). Overall, there were 24 significantly enriched GO terms obtained from all three categories, which collectively showed the functionally coherent network of targets mainly involved in kinase signalling pathways, membrane action, and neurotransmitter receptor regulation. Positive regulation of cell population growth was shown to have the most significant enrichment within the Biological Process category ($-\log_{10}p \approx 3.3$). Additionally, positive modulation of the MAPK cascade ($-\log_{10}p = 2.7$) and phosphatidylinositol 3-kinase/protein kinase B signal transduction were discovered. Both of which were known previously for their importance in neuronal sensitization and inflammatory pain signalling mechanisms. Another significant enrichment in Biological Processes included that for response to xenobiotic stimulus ($-\log_{10}p \approx 2.7$), which may suggest that the targets of koumine might have some involvement in the mechanisms responsible for cellular stress response and related processes like drug metabolism and neuroprotection. Of particular interest was erythropoietin-mediated signalling pathway, whose extremely high fold enrichment (~ 500) suggests that even though fewer targets are associated with this category, they all play very specific roles within this pathway. This is especially interesting in light of recent evidence about neuroprotection by erythropoietin in peripheral nerve injury models. With regards to the Cellular Component category, the term "plasma membrane" was found to be significantly enriched ($-\log_{10}p \approx 3.3$, count = 7). The result means that the proteins that koumine binds to have mostly been localized on membranes; it is also consistent with their classification as receptor tyrosine kinase and transmembrane signalling receptors. The second group of functionally relevant findings are those related to the enrichment of "postsynaptic membrane" ($-\log_{10}p \approx 2.7$) and "glutamatergic synapse" ($-\log_{10}p \approx 2.1$) categories; both are highly important from the point of view of neuropathic pain. Postsynaptic glutamatergic signalling, which takes place within spinal cord synapses, is responsible for central sensitization phenomena. In the category of Molecular Function, protein tyrosine kinase activity ($-\log_{10}p \approx 3.3$, count = 4) and identical protein binding ($-\log_{10}p \approx 3.0$, count = 5) were the two highest-ranked enriched terms, highlighting the importance of phosphorylation through kinases for koumine activity. In particular, serotonin binding displayed the greatest fold enrichment among all MF terms (~ 500 , $-\log_{10}p \approx 2.7$), indicating its specific interaction with serotonergic receptors as a key feature of its pharmacology – in agreement with the high enrichment of the serotonergic synapse pathway

identified by KEGG analysis, as well as the involvement of serotonin signalling in pain modulation. Other enriched terms that support the involvement of kinase-mediated phosphorylation include ATP binding ($-\log_{10}p \approx 2.4$, count = 4), protein kinase activity ($-\log_{10}p \approx 2.4$), and nucleotide binding. Additionally, the enrichment of SH2 domain binding (fold enrichment ~ 200) highlights the importance of protein-protein interactions specific to tyrosine kinase signalling. Taken together, it can be seen from the GO term enrichment analysis that koumine acts at multiple sites that form a functional network of kinases and receptors present in cell membranes, converging primarily on PI3K-Akt/MAPK signalling, postsynaptic glutamatergic transmission, and serotonergic modulation.

S. No	GO Term	Fold Enrichment	$-\log_{10}(p)$	Count
1.	Positive regulation of cell population proliferation	~ 5	~ 3.3	2
2.	Positive regulation of PI3K/PKB signal transduction	~ 5	~ 2.7	2
3.	Positive regulation of MAPK cascade	~ 5	~ 2.7	2
4.	Response to xenobiotic stimulus	~ 5	~ 2.7	2
5.	Erythropoietin-mediated signaling	~ 500	~ 2.7	2
6.	Positive regulation of cell migration	~ 5	~ 2.4	2
7.	Positive regulation of platelet aggregation	~ 350	~ 2.4	2
8.	Behavioral response to cocaine	~ 200	~ 2.4	2

Table 3 : Gene Ontology (GO) enrichment for Biological Process (BP). The table shows the most significant enriched GO terms along with their fold enrichment, p-value (in $-\log_{10}$ format), and gene number.

S. No	GO Term	Fold Enrichment	$-\log_{10}(p)$	Count
1.	Plasma membrane	~5	~3.3	7
2.	Membrane raft	~5	~2.7	3
3.	Postsynaptic membrane	~5	~2.7	3
4.	Focal adhesion	~5	~2.4	3
5.	Membrane	~5	~2.4	5
6.	Endomembrane system	~5	~2.4	4
7.	Glutamatergic synapse	~5	~2.1	3
8.	Cell surface	~5	~2.1	2

Table 4 : Gene Ontology (GO) enrichment for Cellular Component (CC). The table shows the most significant enriched GO terms along with their fold enrichment, p-value (in $-\log_{10}$ format), and gene number.

S. No	GO Term	<u>Fold Enrichment</u>	<u>$-\log_{10}(p)$</u>	<u>Count</u>
1.	Protein tyrosine kinase activity	~10	~3.3	4
2.	Identical protein binding	~10	~3.0	5
3.	Serotonin binding	~500	~2.7	2
4.	Protein tyrosine kinase activator activity	~400	~2.4	2
5.	ATP binding	~5	~2.4	4
6.	Protein kinase activity	~5	~2.4	3
7.	SH2 domain binding	~200	~2.1	2
8.	Nucleotide binding	~5	~2.1	3

Table 5 : Gene Ontology (GO) enrichment for Molecular Function (MF). The table shows the most significant enriched GO terms along with their fold enrichment, p-value (in $-\log_{10}$ format), and gene number

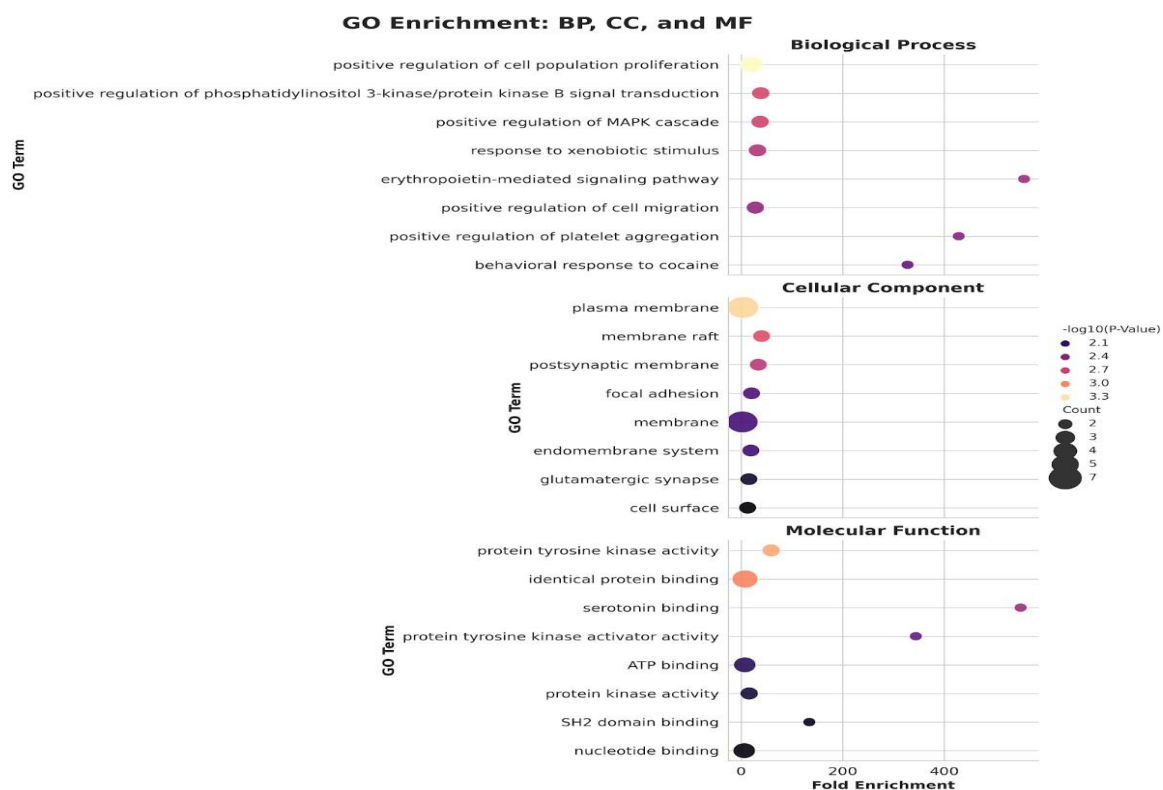


Figure 5: Koumine Target Genes in Neuropathic Pain: Gene Ontology (GO)

4.6 Molecular Docking:

Docking of koumine on the four potential hub targets resulted in good binding energies for all targets (Table 1.4). Of the four hub targets considered, SLC6A4 was noted to have the best binding energy (-9.011 kcal/mol) implying a strong interaction between koumine and the protein; this can be explained by certain interactions that may be occurring inside the serotonergic transporter. The second highest binding energy was noted in KIT (-7.736 kcal/mol), then ABCB1 (-6.606 kcal/mol) and finally EGFR (-6.510 kcal/mol). All binding energies were found to be negative and less than -6.0 kcal/mol hence signifying stability of the interactions between koumine and the hub targets.

S. No	Ligand	Target	Binding Affinity(kcal/mol)
1.	Koumine	KIT	-7.736
2.	Koumine	EGFR	-6.510
3.	Koumine	SLC6A4	-9.011
4.	Koumine	ABCB1	-6.606

Table 6: Showing Docking Scores for the top identified Hub-genes in Neuropathic pain

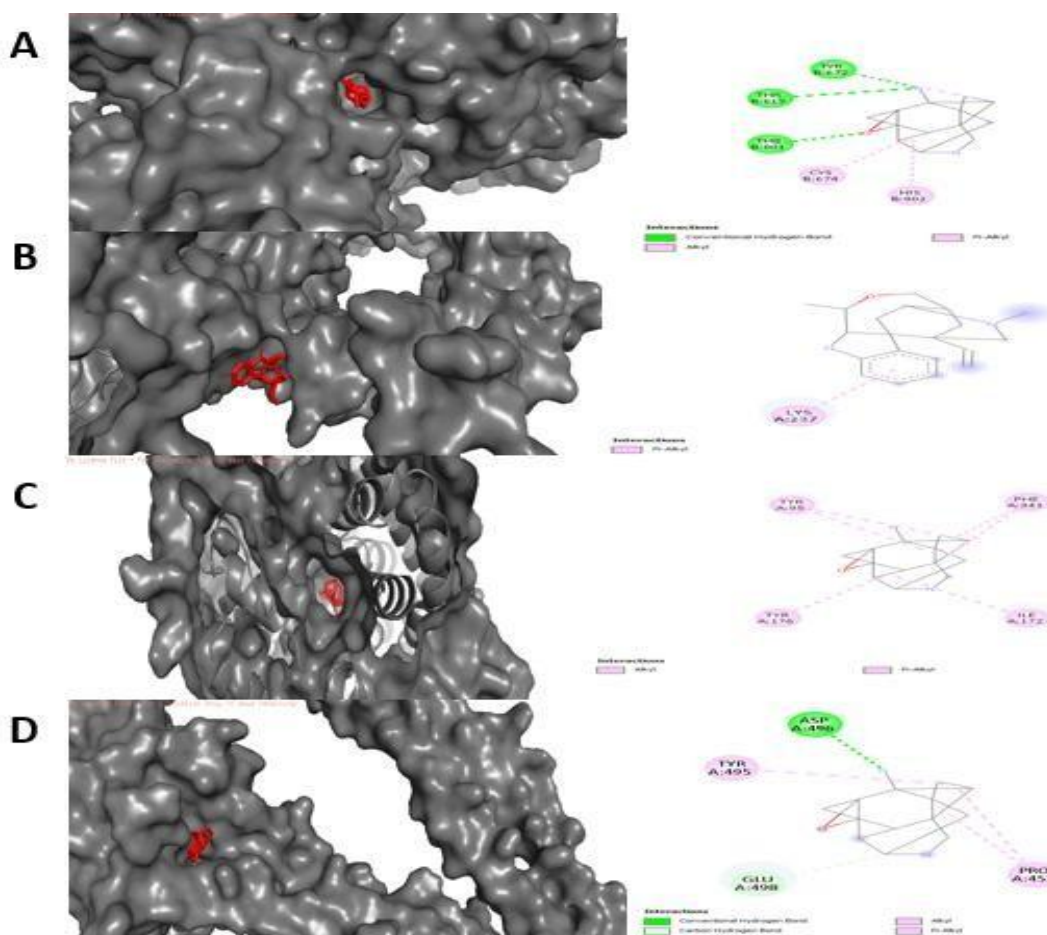


Fig: Molecular Docking results for hub genes A-KIT , B-EGFR , C-SLC6A4 , D-ABCB1

4.7 ADMET Analysis:

Koumine's ADMET study showed advantageous pharmacokinetic characteristics. Good oral bioavailability was indicated by the compound's substantial gastrointestinal absorption. Additionally, it was expected that koumine would be able to cross the blood-brain barrier, a crucial feature for drugs that target neuropathic pain and central nervous system disorders. Furthermore, the molecule satisfied Lipinski's rule of five, indicating good drug-likeness and suitability as a potential therapeutic agent. However, koumine demonstrated favourable results for hepatotoxicity and AMES toxicity, suggesting possible issues based on toxicity forecasts. These results imply that although koumine exhibits encouraging pharmacological characteristics, additional experimental validation is necessary to verify its safety profile. Although koumine's anticipated toxicity emphasizes the need for cautious dose adjustment and in vivo research, the ADMET profile generally supports the drug's potential as a therapy for neuropathic pain.

Table 7: ADMET analysis of Koumine showing its various properties

Category	Parameter	Value
Absorption	GI Absorption	High
	Caco2 permeability	1.507
	Human intestinal absorption	98.058%
	P-glycoprotein substrate	Yes
	P-glycoprotein I/II inhibitor	Yes / No
	Water Solubility (Log S)	-4.023
Distribution	BBB permeant	Yes
	VDss (human)	1.589
	Fraction unbound (human)	0.159
Metabolism	CYP3A4 Substrate	Yes
	CYP3A4 Inhibitor	No
	CYP2D6 Inhibitor	Yes
	CYP2C19 Inhibitor	No / Yes
	CYP1A2 Inhibitor	Yes / No
Excretion	Total Clearance	1.034 log ml/min/kg
	Renal OCT2 substrate	Yes
Drug-Likeness	Lipinski Rule	Yes; 0 violation
	Molecular Weight	306.40 g/mol
	Log P _{o/w} (Consensus)	2.85
	H-bond Donors	0
	H-bond Acceptors	3
	TPSA	24.83 Å ²
	Rotatable Bonds	1
	Bioavailability Score	0.55
	Synthetic accessibility	5.17

Toxicity	AMES toxicity	Yes
	Hepatotoxicity	Yes
	hERG II inhibitor	Yes
	Oral Rat Acute Tox	3.062 mol/kg
	Skin Sensitisation	No

Discussion

The somatosensory nervous system is affected by neuropathic pain. It is caused due to its injury or dysfunction, and it is marked by such symptoms as hyperalgesia, allodynia, and spontaneous pain. The complex pathogenesis of neuropathic pain encompasses such mechanisms as neuroinflammation, oxidative stress, neuronal sensitization, and neurotransmission disorders, making traditional single-target treatments ineffective [44]. As a result, multi-target drug discovery became a novel direction in treating neuropathic pain. In the current paper, a multi-target pharmacological role of Koumine as a treatment agent for neuropathic pain was examined using network pharmacology and molecular docking approaches. It has been found that Koumine is involved in several protein-protein interactions and pathways related to inflammation, neurons' survival, neurotransmission, and pain regulation. Thus, natural compounds may exhibit multi-target pharmacology [45].

The network pharmacology revealed the presence of several vital hub targets, namely JAK2, EGFR, KIT, ABCB1, SLC6A4, HTR2A, and SIGMAR1. The abovementioned genes participate in signalling and functioning of systems related to inflammation, neuron activity, neuroprotection, and sensitization. For example, JAK2 mediates cytokine-mediated inflammatory signalling via JAK/STAT pathway activation and is involved in progression of neuroinflammation and neuropathic pain. The target of EGFR and KIT is involved in cellular signalling, neuronal healing, and regulation of inflammation. Receptors' activation leads to changes in neurons' excitability and chronic pain development [46]. The gene related to the blood-brain barrier's permeability, i.e., ABCB1, can influence the pharmacokinetics of drugs and their central nervous system accessibility [47]. Moreover, the genes of SLC6A4 and HTR2A take part in regulation of serotonergic neurotransmission and control of pain perception. Changes in the pathways of serotonin signalling are directly related to neuropathic pain and development of emotional disturbances due to the latter [48]. Finally,

SIGMAR1 is considered one of the essential neuroprotective regulators involved in calcium signalling, neurons' excitability and regulation of inflammatory response [49]. The above-mentioned targets indicate the ability of Koumine to affect

Several key biological pathways such as PI3K-Akt, MAPK, TNF, NF-kappa B, and neuroactive ligand-receptor interaction were significantly enriched according to the KEGG pathway analysis. The aforementioned pathways have been reported to be of great significance in the process of inflammation, neuronal survival, synaptic signalling, and sensitization leading to pain in the case of neuropathic pain development. In this respect, PI3K-Akt and MAPK signalling pathways have already been stated to be crucial to neuronal protection and central sensitization, while TNF and NF-kappa B signalling pathways are involved in the regulation of inflammatory[50], [51]. Thus, it may be assumed that Koumine alleviates pain during the formation of neuropathic pain by altering multiple signalling pathways simultaneously. Additionally, Gene Ontology enrichment showed that there was also a significant participation of protein tyrosine kinases activity, transmembrane receptor signalling pathway, and MAPK cascade regulation. It is crucial to remember that the enrichment of tyrosine kinase-related pathways may suggest the possible regulatory effect of Koumine in relation to the receptors' pathways linked to sensitization and neuroinflammation. Another interesting point was that the significant intersection of Koumine targets was in relation to MAPK signalling and protein tyrosine kinases activity. In addition, this current study has shown enrichment of biological processes involved in lipid metabolism, atherosclerosis, and central carbon metabolism. Current evidence suggests that oxidative stress, metabolic imbalance, and systemic inflammation are important contributors to the emergence of neuropathic pain. This implies that Koumine might have multiple biological effects that extend beyond the classical pain pathways. The molecular docking study provided further proof to support the results obtained using network pharmacology in terms of stable interaction of Koumine with key proteins like EGFR, KIT, JAK2, and SIGMAR1. The high binding affinity revealed by the docking experiment indicated that Koumine is capable of regulating the downstream signalling pathway associated with inflammatory and neurochemical reactions [52].

The results found in the current investigation coincide with those obtained in pharmacological studies, which indicate that Koumine has both analgesic and anti-inflammatory activity along with neuroprotective effect. According to earlier research, koumine reduces the generation of inflammatory cytokines that cause pain hypersensitivity and suppresses neuro-inflammation [53]. The results of the current study are expanded by using computational techniques to analyze koumine's pharmacological action mechanism on several targets. Other than the target

and pathway analysis, ADMET predictions also showed that Koumine had a promising pharmacokinetics profile. Koumine was found to have adequate intestinal absorption, drug-likeness as per the Lipinski rule of five, as well as the ability to pass the blood-brain barrier – an important feature of drugs intended for neuropathic pain and diseases related to the central nervous system[54]. Nevertheless, the toxicology study suggested the possibility of hepatotoxicity and AMES toxicity, making the dose optimization and testing necessary.

However, despite these interesting results, there are some drawbacks related to the current study. It uses a majority of computations which are based on publicly accessible databases and computational algorithms. Molecular docking does not fully consider flexibility of the protein in its interactions with ligands since it gives a picture that is static in nature [55]. In addition to this, the signalling pathways and target proteins predicted by the study have not been confirmed by any experimental approach in either an in vitro or in vivo setting. As seen above, a combination of network pharmacology, molecular docking, and ADMET analysis provides us with a thorough understanding of Koumine's multiple-targeted mode of action as a neuropathic pain treatment alternative. These findings imply that Koumine might be able to relieve neuropathic pain by regulating inflammatory pathways, neurotransmitters, and neuroprotection. To validate the anticipated effects and determine the potential therapeutic use of Koumine as a multitargeted medication for neuropathic pain treatment, additional study is required.

CHAPTER 5

CONCLUSION AND FUTURE PERSPECTIVE

The current study assessed the potential therapeutic benefit and underlying mechanism of action of Koumine on neuropathic pain using an integrated network pharmacology and molecular docking analysis. In light of the fact that neuropathic pain is a complicated disease in which several factors play critical roles, it becomes necessary to look for multi-target drugs that can be effective against such a condition.

According to the results obtained from this study, Koumine acts via a multi-target drug mechanism by targeting a range of molecules related to neuropathic pain, including JAK2, EGFR, KIT, ABCB1, SLC6A4, HTR2A, and SIGMAR1. Such molecules are related to neuroinflammation, neuronal signalling, neurotransmission, neuroprotection, and pain sensitization, implying that Koumine could act on several biological pathways of neuropathic pain pathogenesis. PI3K-Akt, MAPK, TNF, NF-kappa B, and neuroactive ligand-receptor interaction pathways were among the pathways shown to be enriched in the KEGG pathway enrichment analysis. These pathways are known to be related to inflammation, neuron survival, synaptic plasticity, and chronic pain progression. It can be concluded that Koumine acts on neuropathic pain not by acting on a singular molecule but through the modification of several pathways. Molecular docking study further proved the results obtained from the network pharmacology approach by showing good binding affinity between Koumine and proteins including EGFR, KIT, JAK2, and SIGMAR1. Moreover, ADMET analysis has shown good pharmacokinetics of Koumine, including absorption from the gut, blood-brain barrier passage, and drug-like property parameters. On the other hand, predicted hepatotoxicity and AMES toxicity mean that more toxicological analysis is needed.

In summary, the current study offers an integrated systems biology perspective for elucidating multi-targets and multi-pathways involved in Koumine for treating neuropathic pain. Although the study was successful, it should be emphasized that the results were primarily based on in silico analysis; therefore, additional experimental investigation will be required. The results indicate that Koumine can function as a possible therapeutic candidate for neuropathic pain management. Even though the study was successful, it should be highlighted that more experimental analysis will be required because the outcomes of the current research were primarily based on in silico analysis.

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