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Research Article
Network Pharmacological Investigation of Sinomenine Action Against Synovitis

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Abstract

Background and Objective: Synovitis is characterized as the inflammation of the synovial membrane, which often occurs in osteoarthritis. The incidence of this medical condition may be related to age, immunological responses and other co-morbidities. Therefore, the objective of the study was to elucidate the anti-Synovitis potential of sinomenine. **Materials and Methods:** The associated targets about sinomenine and synovitis were investigated in *Homo sapiens*, which was then elucidated by the PPI network construction via STITCH database. Furthermore, Cytoscape and its plugin were used for gene ontology (GO) analysis. **Results:** The literature survey and the network revealed 25 potential target proteins to be associated with sinomenine, of which many proteins such as OPRD1, CHRM1 and DAMGO were found to be significantly related to the bioactive action of sinomenine in *Homo sapiens*. Moreover, the GO terms which were associated with the functioning of sinomenine for its anti-Synovitis potential were found to be four, analyzed by the functional annotation gene clusters and abundance values of the target proteins. **Conclusion:** The results of the study demonstrate the anti-Synovitis potential of sinomenine, where its action is dependent upon the molecular mechanisms that exert its beneficial role against synovitis. The core mechanisms that may be related to its anti-synovitis action may be adenylate cyclase-activating G-protein coupled receptor signalling pathway, regulation of smooth muscle contraction, adenylate cyclase-inhibiting G-protein coupled acetylcholine receptor signalling pathway and positive regulation of nitric oxide metabolic process.

Key words: Sinomenine, synovitis, STITCH, ClueGO, mode of action, molecular targets, DAMGO, OPRD1

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Synovitis is characterized as the inflammation of the synovium which elicits pain upon movement, usually attributed to the collection of fluid (effusion) in the synovial sac. This condition appears in patients suffering from osteoarthritis, where its histological features are characterized by vascularization, hyperplasia and fibrosis patients[1]. Moreover, the ingress of leukocytes from the vascular tissues regarding the release of cytokines and attachment molecules[2] is observed in synovitis. Many studies have demonstrated that T cells and macrophages are the most prominent immune cells in the synovial sac during the manifestation of osteoarthritis, whereas, B cells and mast cells are also found but in fewer amounts[3-5]. Synovitis has been identified as a common feature in osteoarthritis, where it has been routinely identified by various imaging techniques. Additionally, many researchers have opted for score systems that semi-quantitatively improve the concept of identifying and characterizing synovitis in osteoarthritis affected patients[6]. In extensively movable joints such as the knee, synovitis is significantly related to pain and inflammatory factors[7,8]. Furthermore, it has been related to joint function and movability, which ultimately affects walking and strenuous activities such as climbing stairs in patients[9]. The relativity of knee pain and joint pathogenesis was detected by MRI in patients suffering from knee osteoarthritis in a study, which also correlated with the clinical findings of lesions, tears and inflammation[10]. Many pathway mediators are involved in the development and pathophysiology of synovitis, with IL and NF-κB being the primarily reported mediators, with the latter being involved in the release of pro-inflammatory cytokines that contribute to localized damage to tissues and organs[11,12]. Tumour Necrosis Alpha (TNF) and ILs have been reported to be elevated in the synovium during osteoarthritis, signalling increased inflammation[13].

Traditional Chinese medicine (TCM) has harboured a running streak in the management of clinical acute and chronic pain, due to the potentiality of a vast collection of herbal drugs and compounds. The presence of pharmacological compounds such as sinomenine in herbal plants exhibits few side effects as compared to the therapeutic agents used in the West. This approach has garnered the attention of scientists and researchers towards TCM for the exploration of their advantages for the development of therapeutic healthcare[14,15]. Since its introduction as a systems-biology application, network pharmacology is regarded as a potential aspect of predicting drugs and their related target proteins[16]. It also reveals the complex understanding of several herbs and their compounds and their disease interactions which are very much likely to present new findings, defining the multi-target and synergistic action of many herbs and compounds found in TCM[17].

Sinomenine, a compound purified from the plant *Sinomenium acutum*, had been primarily associated since the 1930s with the treatment of rheumatism in Japan[18]. Its biological activity has been reported in many *in vitro* and *in vivo* studies, with potential protective abilities against many medical conditions and diseases, including inflammatory disorders[19]. It is reported to function as a suppressor of the immune system as it leads to the inhibition of the lymphocyte production and other antibodies in mammalian cells[20,21]. In China, it has been used in many formulations and drugs which are used as a therapy against rheumatoid arthritis. Sinomenine has also been reported in many studies to greatly reduce the onset and management of chronic and acute pain, with it being more effective in reducing the stiff and painful joints in RA patients[22]. Additionally, sinomenine was also found to be effective in reducing neuralgic pain[23]. Various studies have also demonstrated efficacious properties of sinomenine via the increase in overall pain threshold in much experimental pain models[24-28]. In this study, the potential of sinomenine in the management of synovitis was explored by using a network pharmacology approach. At present, no elucidative study marks the explicative mechanism of action of sinomenine in managing Synovitis. Therefore, its detailed mechanism of action, the associated biological pathways and its interaction between multiple proteins remain to be elucidated to confirm its effective role in managing synovitis. Therefore, it is necessary to investigate the said mechanism of action, with specific attention given to the multi-target interaction of sinomenine. Therefore, a network pharmacology approach has opted for this study, where the various biological pathways and their interactions with drugs help to determine the predicted action of sinomenine in managing Synovitis.

MATERIALS AND METHODS

Study area: This network pharmacological study was performed from 1st of January to December 31st, 2020 in the Lab of In Silico Technologies, Department of Sports Medicine, Dalian Central Hospital Affiliated to Dalian Medical University, Liaoning Dalian, 116033, China.

The role of sinomenine in managing Synovitis was investigated by using the STITCH database in the first step of network analysis, which consisted of a systematic study. The second step involved the construction and analysis of a network of sinomenine and its associated protein networks. Furthermore, GO enrichment analysis was conducted using Cytoscape to elucidate the molecular action of sinomenine against Synovitis in Fig. 1.

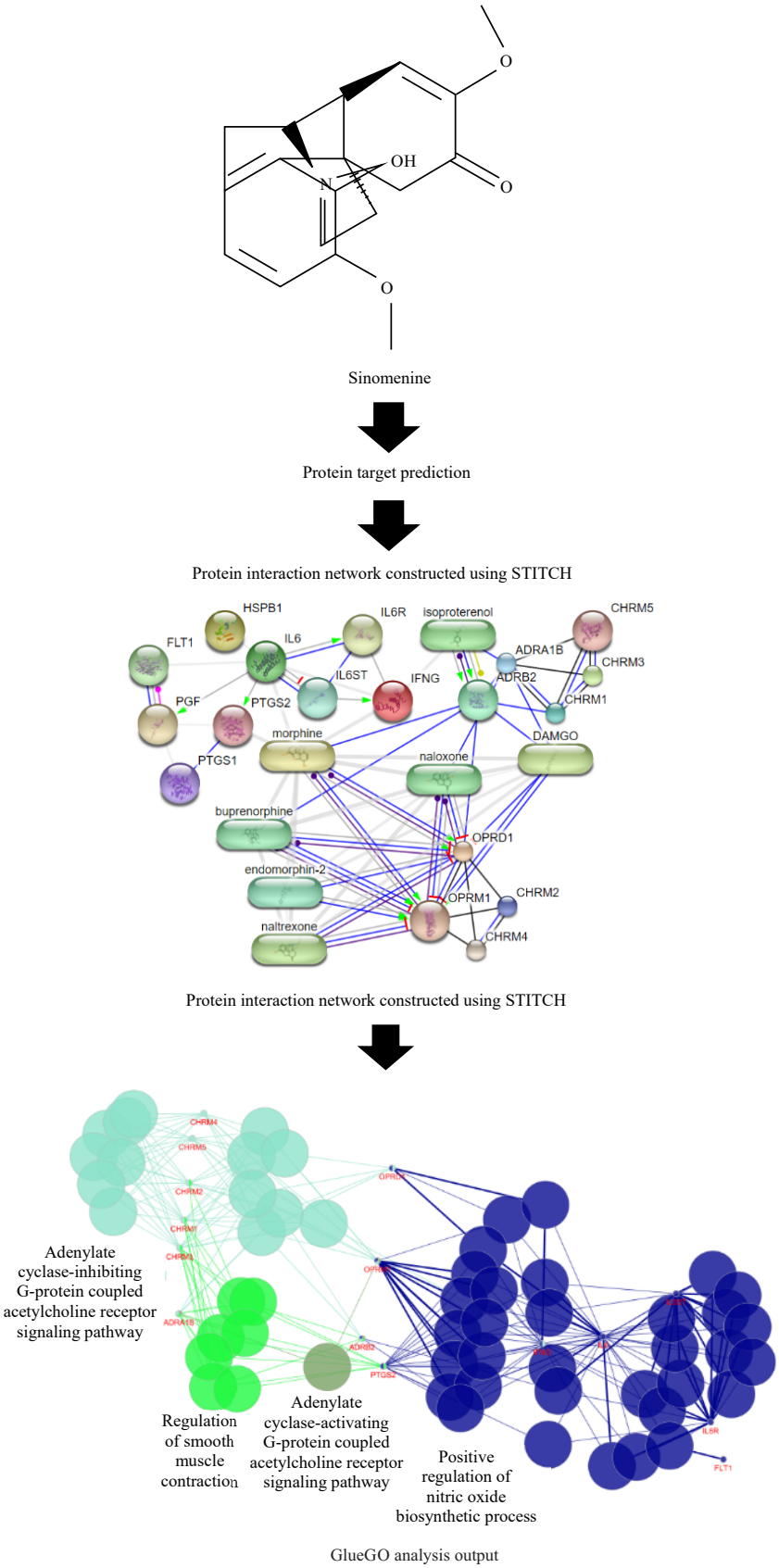

Fig. 1: Flow chart presentation of systematic procedure

Retrieval of sinomenine and its targets: This step was performed by screening the target proteins of sinomenine on the SymMap database[29], which was accompanied by extracting relevant genes from the UniProtKB database. The KEGG database was then utilized for the screening of the association of these proteins with urinary incontinence, after which they were selected for further study.

Network construction and analysis: The interaction of sinomenine and its related protein targets was investigated using STITCH database[30], which is a comprehensive online database containing the knowledge of more than 2000 organisms and around 10 M proteins and their interactions. To investigate the interaction of sinomenine with its target proteins, the PPIN was constructed and analyzed.

Gene ontology and pathway enrichment analysis: The protein targets of sinomenine were revealed by Gene Ontology enrichment analysis, which was performed by using ClueGO, a plugin for Cytoscape[31,32], with the significance level set down at 0.05. Medium network, two-sided hypergeometric test with Bonferroni correction were the parameters that were set for the analysis. Eventually, the functional network was analyzed and envisaged using the algorithmic organic layout.

RESULTS

Retrieval of sinomenine features: The significant features of sinomenine (MOL012920) as a compound were retreived as follows: MW = 329.43, AlogP = 1.32, Hdon = 1, Hacc = 5, OB (%) = 30.98, Caco-2 = 0.62, BBB = 0.43, DL = 0.46, FASA- = 0.21, TPSA = 59.00, RBN = 2 and HL = 1.79.

Retrieval of sinomenine targets: From the SymMap database, 25 potential targets of sinomenine were found, which according to the literature reported, could be utilized to manage synovitis and were associated with the management of pain. Subsequently, these targets were screened and standardized through the UniProt database system. Out of these 25, 18 potential target proteins were reported to be found in *Homo sapiens* in Table 1. Most of these target proteins are linked with prostaglandin synthesis and muscarinic acetylcholine receptors. The influence of muscarinic acetylcholine receptors could be excitatory and inhibitory. Muscarinic receptors do affect the smooth and cardiac muscles and exocrine glands.

Table 1: TCMSP-based retrieval of protein target database

No.	Protein names
1	Prostaglandin G/H synthase 1
2	Muscarinic acetylcholine receptor M3
3	Muscarinic acetylcholine receptor M1
4	Sodium channel protein type 5 subunit alpha
5	Muscarinic acetylcholine receptor M5
6	Prostaglandin G/H synthase 2
7	Muscarinic acetylcholine receptor M4
8	Retinoic acid receptor RXR-alpha
9	Delta-type opioid receptor
10	Sodium-dependent noradrenaline transporter
11	Muscarinic acetylcholine receptor M2
12	Alpha-1B adrenergic receptor
13	Beta-2 adrenergic receptor
14	DNA topoisomerase II
15	Mu-type opioid receptor
16	Heat shock protein HSP 90
17	Neuronal acetylcholine receptor protein, alpha-7 chain
18	Interleukin-6
19	Heat shock protein beta-1
20	Interleukin-2
21	Interferon gamma
22	Placenta growth factor
23	Death domain-associated protein 6
24	Programmed cell death 1 ligand 1
25	Programmed cell death 1 ligand 2

Network construction and analysis: An elaborate PPI network was developed via the STITCH database, with 18 target proteins having a confidence score of 0.400 and with 18 nodes and 29 edges. The resultant PPI network is comprised of functional interactions, where the nodes and edges represented the target proteins and/or their associated genes and the interactions between different genes, respectively. Details of the constructed network demonstrated its p-value to be negligibly small (2.4e-08), whereas, the number of edges for the network was found to be 1 at randomly chosen nodes. Small p-value denotes the non-random selection and the significant number of edges in a network, respectively. In this PPI network, the values for node degree and clustering coefficient were found to be 3.22 and 0.81, respectively. Moreover, 8 functional hubs were reported in the PPI network due to their nodal degree being greater than the average nodal degree, where node degree = 3.22. In this case, OPRD1 protein possessed the highest degree = 10. The subsequent proteins were observed to be CHRM1 and DAMGO, each having 9 node degrees, respectively in Table 2. These findings show that OPRD1, CHRM1 and DAMGO proteins are widely associated with other proteins in the body, forming a protein-protein interaction network and likely play a significant role in various pharmacological activities.

Table 2: Node degree of sinomenine targets obtained through STITCH

Node	Node degree	Node	Node degree
IFNG	5	PTGS2	4
OPRD1	10	CHRM5	1
HSPB1	1	OPRM1	4
CHRM3	3	CHRM4	2
IL6	8	PGF	4
ADRB2	7	IL6R	3
CHRM1	9	DAMGO	9
ADRA1B	7	FLT1	2
CHRM2	3	Endomorphin-2	6
PTGS1	2	IL6ST	3

Table 3: Functional protein targets obtained through STITCH analysis

Proteins	Activation	Inhibition	Binding	Score
IL6R	●		●	0.999
DAMGO	●		●	0.999
FLT1	●		●	0.999
Endomorphin-2	●	●	●	0.999
IL6ST	●	●	●	0.999

●: Presence

The action view of the functional nodes is provided in Table 3, demonstrating the several functions of the proteins, providing an overview of the various roles of the protein targets, such as activation, inhibition and binding, respectively. Mechanistically, sinomenine bind with and activate all the retrieved protein targets, i.e., IL6R, DAMGO, FLT1, endomorphin-2 and IL6ST, however, Endomorphin-2 and IL6STare inhibitory as well by the sinomenine. These interactions mainly result in improved immunity.

Table 4 describes the functional enrichments of the biological processes, molecular functions, cellular components and KEGG pathways in the protein-protein interaction network. Most of these elements (either process, function or pathway) have association with neurotransmitter receptor activity and G-protein coupled receptors. All the retrieved biological process related GO terms (i.e., 7197, 7200, 7207, 7188 and 7193) are mainly associated with G-protein coupled receptors. These GO terms consist of a variable number of genes, ranging from 5 to 9. The STITCH uses algorithms to apply false discovery rate (FDR) on the associated constituents in the gene set network. The retrieved GO terms had FDR values in a range of 2.36e-09-5.9e-11. It suggests that the observed biological processes have significantly higher occurrence with the use of sinomenine than without using it. Both muscarinic acetylcholine receptors and G-protein coupled receptors are involved in the parasympathetic nervous system. These receptors are mainly involved in bronchoconstriction, peristalsis and micturition. The stimulation of the parasympathetic nervous system of cardiac muscarinic receptors commonly results in diminished contractility and AV nodal contraction.

In addition, the retrieved molecular functions are also mainly associated with G-protein coupled receptors. The number of genes associated with the obtained GO terms and the relevant FDR valesare5-12 genes and <0.05, respectively. These findings support the mode of the above-documented biological effects of sinomenine. Furthermore, cellular components are related to synapses and plasma membranes and their role in neuronal conductivity, for instance, asymmetric synapse which is excitatory in function. Such kind of cellular components has a significant contribution to molecular pathways involved in managing synovitis. Here also, the obtained GO terms and the relevant FDR values are 3-14 genes and <0.05, respectively. Lastly, KEGG pathways are also associated with neuronal signalling pathways, such as the PI3K-Akt signalling pathway and cholinergic synapse. These pathways have involvement in cholinergic gene expression. Most of the above findings have revealed a significant effect of sinomenine on various biological and molecular processes, cellular components, or signalling pathway which are related to cartilage degradation, synovial inflammation and subchondral bone dysfunction.

Gene ontology and pathway enrichment analysis: The evaluated protein targets of sinomenine were investigated via ClueGO, for its pathway mediated enrichment analysis. The major enrichment was found to be of 28 GO terms, which were categorized into 9 sub-groups. These sub-groups were mainly involved in adenylate cyclase-activating G-protein coupled receptor signalling pathway (GO ID 7189), regulation of smooth muscle contraction (GO ID 6940), adenylate cyclase-inhibiting G-protein coupled acetylcholine receptor signalling pathway (GO ID 7197) and positive regulation of nitric oxide metabolic process (GO ID 1904407) in Table 5. The p-values of the retrieved GO terms and clusters and their p-values (corrected with Bonferroni step down) are less than 0.05, showing their significantly higher occurrence with the use of sinomenine than without using it. The genes associated with the retrieved GO terms are mainly adrenergic receptors (ADR) and cholinergic receptors muscarinic (CHRM). CHRM forms G-protein-coupled receptor complexes in the nerve cell membranes, which are sensitive to the muscarine. Stimulation of CHRM mediates important physiological functions including smooth muscle contraction. Targeting CHRM is a useful approach for various inflammatory conditions. ADR play a role in the inflammatory response of the synovial fibroblasts. Thus, ADR modulation exhibit a therapeutic strategy to modulate synovial fibroblast function in synovitis. These findings provide significant support in the elucidation of the anti-Synovitis role of sinomenine.

Table 4: Functional enrichments in network

Pathway ID	Pathway description	Count in gene set	False discovery rate
Biological process (GO)			
GO:0007197	Adenylate cyclase-inhibiting G-protein coupled acetylcholine receptor signalling pathway	5	2.46e-11
GO:0007200	Phospholipase C-activating G-protein coupled receptor signaling pathway	8	5.9e-11
GO:0007207	Phospholipase C-activating G-protein coupled acetylcholine receptor signalling pathway	5	5.9e-11
GO:0007188	Adenylate cyclase-modulating G-protein coupled receptor signalling pathway	9	1.86e-10
GO:0007193	Adenylate cyclase-inhibiting G-protein coupled receptor signalling pathway	7	2.36e-09
Molecular function (GO)			
GO:0016907	G-protein coupled acetylcholine receptor activity	5	7.32e-12
GO:0008227	G-protein coupled amine receptor activity	7	2.37e-10
GO:0030594	Neurotransmitter receptor activity	6	1.7e-07
GO:0004888	Transmembrane signalling receptor activity	12	8.59e-07
GO:0004930	G-protein coupled receptor activity	9	4.87e-05
Cellular component (GO)			
GO:0032279	Asymmetric synapse	4	3.56e-07
GO:0005887	Integral component of plasma membrane	13	1.05e-06
GO:0005896	Interleukin-6 receptor complex	3	1.05e-06
GO:0044459	Plasma membrane part	14	8.4e-06
GO:0045211	Postsynaptic membrane	6	3.25e-05
KEGG pathways			
04080	Neuroactive ligand-receptor interaction	9	5.02e-09
04020	Calcium signalling pathway	6	7.72e-06
04725	Cholinergic synapse	5	1.67e-05
04151	PI3K-Akt signalling pathway	6	0.000183
04810	Regulation of actin cytoskeleton	5	0.000268

Table 5: GO terms, their IDs and associated genes involved in the action of sinomenine

GO-ID	GO Term	Term p-value	Term p-value corrected with Bonferroni step down	Group p-value	Group p-value corrected with Bonferroni step down	Associated genes found
7189	Adenylate cyclase-activating G-protein coupled receptor signalling pathway	5.10E-05	1.02E-04	5.10E-05	5.10E-05	ADRA1B, ADRB2, OPRM1
6940	Regulation of smooth muscle contraction	1.71E-11	6.16E-10	1.40E-08	2.80E-08	ADRA1B, ADRB2, CHRM1, CHRM2, CHRM3, PTGS2
7197	Adenylate cyclase-inhibiting G-protein coupled acetylcholine receptor signaling pathway	6.49E-16	2.86E-14	1.14E-12	4.54E-12	CHRM1, CHRM2, CHRM3, CHRM4, CHRM5
1904407	Positive regulation of nitric oxide metabolic process	1.28E-07	3.97E-06	1.37E-08	4.12E-08	IFNG, IL6, OPRM1, PTGS2

DISCUSSION

Chinese medicine has extensively employed the use of many herbs and compounds for the treatment and management of many medical ailments and diseases. These herbs work or exert their pharmacological activity via the action of bioactive compounds, or the active ingredients of the TCM herb pharmacopeia. Sinomenine, another invaluable compound in use for centuries, has a wide range of therapeutic applications, including synovitis. This study employs a predictive approach in deciphering the potential of sinomenine in treating Synovitis. The study used a multi-step approach, which involved the construction of a PPI network and enrichment analysis of proteins that were associated with sinomenine.

The PPI network of the predictive study revealed the major proteins in the network to be OPRD1, CHRM1 and DAMGO, which were found to be significantly associated with the biological action of sinomenine against Synovitis. Many researchers have demonstrated the application of opioid compounds and their receptors in the management of pain in arthritis, without revealing some serious side effects. These receptors are majorly classified on the traditional nomenclature of -δ (delta), -κ (kappa) and -μ (mu), where these receptors are primarily categorized into the family of G protein-coupled receptors (GCPR)[33]. Opioids have been a major constant in the management of pain therapy for many years and are a part of many herbal practices and pharmacopeia. These receptors act at various sites of the human body[34] where these receptors cause a wide range of

unwanted side effects, such as sedative effects, euphoria, respiratory depression, nausea and constipation[35,36]. Nevertheless, the activation of the opioid receptors and their associated pathways are attributable to the attenuation of neurons and the hyperexcitability caused by inflammation or trauma[37,38]. This is established by the various studies over the years that validate the use of morphine in osteoarthritis, promoting analgesic effects, anti-inflammatory behaviour, as well as demonstrating effectiveness in bone and joint disorders in humans[39-41]. Delta opioid receptors have been reported to possess analgesic efficiency without the unwanted effects of other receptors used in alternative therapies. However, the efficacy of the delta-opioid receptors depends upon the severity of pain, injury type and the surrounding environment, respectively[42]. Therefore, the ligands exhibit low efficacious potential in the case of acute pain whereas demonstrating remarkable efficacy in the case of chronic pain models, which are often marked by inflammation[43-45].

The pathway enrichment analysis demonstrated that all the analyzed GO terms are relatively associated with the management of synovitis, i.e., adenylate cyclase-activating G-protein coupled receptor signalling pathway, regulation of smooth muscle contraction, adenylate cyclase-inhibiting G-protein coupled acetylcholine receptor signalling pathway and positive regulation of nitric oxide metabolic process.

Pain is a significant yet undesired response to any trauma or injury which can pertain to tissue damage, but can altogether take the shape of a disease or a medical ailment if it persists for a long period, often harbouring physical difficulties[46]. When denoting a disease, pain demonstrates a remarkable burden that is known both socially and economically, respectively. Globally, pain is characterized on various levels and a wide variety of thresholds, demonstrating that there is no gold standard to measuring pain in humans. Every person's threshold to pain tolerance is indeed unique and this calls for specific treatment methods that can be attuned to a specific person and their pain management requirements, respectively[47]. For the management of acute and chronic pain, the most opted methods for several medical conditions remain the use of opioid substances, which attenuate the severity of pain[48].

G-protein coupled receptors (GCPRs) are a family of receptors that mediate pain or analgesia by participating in several molecular mechanisms, as seen evidently from the significant receptor trafficking and specific cellular processes that modulate pain at different thresholds levels[49,50]. Moreover, the downstream or upstream regulation of the GCPR family mediates many biological pathways which are rudimentary in managing pain, inflammation in the case of bone and other ailments. The classic downstream signalling which is mediated by GCPR reckons the active transitioning state of the receptor's binding sites which are then able to bind to several of the members of the GCPR and other proteins. Characteristically, the activation of the GCPR family is mediated by the binding of ligands to the orthosteric binding site which is also responsible for the binding of other endogenous ligands well[48]. The constitutively and spontaneously related processes of GCPRs are well-known to be involved in signal transduction processes[51], which forms the basis of the usage of receptors are basic targets in pharmacological research[52]. The signalling pathways which are mediated by GCPRs are attributable to the functioning of cells and are typically known to mediate (both regulate and inhibit) the classical adenylyl cyclase (cAMP) signalling pathway[53]. This pathway is responsible for relaying the signals from the extracellular to the intracellular environment and vice versa, thus resulting in a cascade of signalling response[54]. Furthermore, G proteins transduce downstream signalling via the stimulation of adenylyl cyclase to produce cAMP which is a significant mediator[55,56]. Many studies have reported the role of GCPRs in the regulation of mediators such as cAMP and their signalling, thus highlighting the crucial role of G proteins in the management of pain and inflammation regulating pathways and biological processes[57,58].

The current study has effectively predicted the previously unforeseen role of sinomenine in the management of synovitis, revealing its potential pharmacological action in managing its various symptoms, as well as activating the different biological processes that aid in mediating and mitigating the related symptoms of pain and inflammation. Nevertheless, this study pertains to the *in silico* approach and is arguably a very preliminary study in its initial phase. Its validation, as well as further insight, can be achieved by advanced studies that can elucidate the exact mechanistic action of sinomenine in treating and managing synovitis.

CONCLUSION

The era of *in silico* analyses and studies has bestowed novel insight into the treatment of various medical ailments by plants and herbs and their bioactive compounds. The prediction of the therapeutic and effective potential of plants has been made unanimously well known by the approach of network pharmacology, which has been altogether very significant in the identification of users and bioactive compounds. The mode of action of TCM in the treatment of synovitis is mediated through the interaction of biologically active compounds with various functional proteins,

accentuating immune responses in human beings as well as regulating the associated biological pathways, respectively. This, coupled with several useful bioinformatics approaches, can aid greatly in deciphering the mode of action of TCM herbs. The use of in silico methods proves to be a resourceful way of discovering the molecular mode of action of various traditionally used herbs, as well as deciphering new functions of established herbs against various progressive diseases.

SIGNIFICANCE STATEMENT

This study proposed the action mode of sinomenine in treating and managing synovitis that can be beneficial for its preclinical trials. This study will help the researchers to uncover the critical areas of synovitis treatment that many researchers were not able to explore. Thus a new theory on the potential role of sinomenine against Synovitis may have arrived at the suggestion of testing the potential of this finding in animal models.

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