ORIGINAL ARTICLE

Understanding Mechanisms of Sinomenine in Morphine Addiction Treatment Using Network Pharmacology and Molecular Docking Approaches

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ABSTRACT

Introduction: Sinomenine, derived from *Sinomenium acutum*, has been reported as a potential treatment for morphine addiction but its mechanisms are poorly understood. Hence this study was conducted to investigate the potential mechanisms underlying sinomenine effects on morphine addiction. **Materials and methods:** Potential protein targets for sinomenine were predicted using SwissTarget Prediction and PharmMapper while morphine addiction targets were collected from DisGeNet and GeneCards databases. Protein-protein interaction was examined using GeneMANIA web server while Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were conducted using ShinyGO online tool. Topological network analysis was performed using Cytoscape to measure the degree centrality, betweenness centrality and closeness centrality values while molecular docking analysis was done using AutoDock Vina to determine the binding energies and interactions. **Results:** A total of 15 sinomenine targets were identified to be involved. Among the identified targets, 37.94% shared protein domains and 19.64% displayed physical interactions. Relevant biological processes, molecular functions, cellular components and signalling pathways were identified involving G-protein coupled opioid receptor signalling pathways and activities, integral component of presynaptic membrane and mitophagy. Molecular docking suggested that the substituted aromatic ring of sinomenine plays important roles in the binding to the protein targets. The top five most significant protein targets were identified based on the binding energies and degree centrality values, namely OPRD1, OPRK1, NOS1, OPRM1 and SRC. **Conclusion:** Sinomenine interacted with various protein targets and pathways which can potentially treat morphine addiction mainly via opioid receptors and their signalling pathways.
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Keywords: Morphine addiction, Sinomenine, Molecular docking, Network pharmacology, Protein targets

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INTRODUCTION

Morphine addiction refers to a condition characterized by a physical and psychological dependence towards the drug (1). The condition significantly impacted the well-being of individuals struggling with addiction and has emerged as a critical societal concern jeopardizing public safety. The World Drug Report revealed that approximately 61.3 million people globally engaged in opioid use, including morphine, in the preceding year of 2020 (2). Presently, addiction to morphine and other illicit substances is acknowledged as a persistent and recurring neurological condition. Substantial evidence indicates that the fundamental aspects of morphine addiction involve the establishment and endurance of addictive memory rooted in morphine-triggered gene expression and alterations in synaptic plasticity (3, 4). It was hypothesized that disrupting this addiction memory most likely will represent a potentially effective strategy for addressing morphine addiction (5). Nevertheless, the specific brain regions and biological mechanisms linked to morphine addiction memories remain largely unexplored due to the complexity of the disease itself where multiple receptor targets and signalling pathways were involved. On top of that, various factors such as biological, psychological and environmental factors also can affect the disease development.

Sinomenine, a natural alkaloid derived from the Chinese medicinal plant *Sinomenium acutum*, has garnered attention for its therapeutic benefits across a

range of ailments, including rheumatoid arthritis, pain and atherosclerosis from various pharmacological investigations (6-8). These positive effects are primarily attributed to its multifaceted pharmacological actions, encompassing anti-inflammatory and antioxidant properties. Beyond its impact on peripheral tissues and organs, sinomenine has demonstrated notable advantages in various animal models of central nervous system (CNS) disorders due to its ability to swiftly traverse the blood-brain barrier (9). Sinomenine is currently recognized as a potential agent for the prevention or treatment of CNS disorders although the lack of precise understanding of its role in such conditions (10).

From pharmacological perspective, the use of sinomenine has been suggested to be advantageous in morphine addiction treatment based on the recent studies (11-13). In these studies where conditioned-place preference paradigm was used to assess the rewarding effect of morphine, sinomenine was able to attenuate the morphine rewarding effects pointing towards its therapeutic potential as a morphine addiction treatment. Despite its chemical structure closely related to morphine, sinomenine did not cause physical dependence like morphine rendering it as a drug with low abuse potential (14). The mechanisms that have been reported involved reduction of tyrosine hydroxylase (TH) and N-methyl-d-aspartate receptor 2B (NR2B) expression, increased mu-opioid receptor (MOR) expression, inhibition of morphine-induced activation of astrocytes and regulation of the homeostasis of gut microbiota (11-13). There are also possibilities that this therapeutic effect of sinomenine is attributed to its anti-inflammatory activities (15) and also neuroprotective effects (16). These properties could contribute to the potential of sinomenine to be developed further for addressing morphine addiction problem.

This study was conducted to elucidate the involvement of potential protein targets and molecular pathways of sinomenine as a treatment for morphine addiction. Network pharmacology and molecular docking approaches has been utilized in many studies seeking to understand the mechanism of action of drugs from the multiple target and multiple pathways perspectives (17). This study employed the abovementioned methods to achieve the objectives of the study where previously the same approaches have been done in elucidating the mechanism of sinomenine to treat allergic rhinitis (18), myocardial injury (19) and ulcerative colitis (20). Molecular docking on the other hand was utilized to predict the favourability of sinomenine to bind to the predicted protein targets and visualize the binding interactions involved. This study aimed towards the expansion of our limited knowledge with regards to the pharmacological effects of sinomenine in morphine addiction treatment.

MATERIALS AND METHODS

Predicting the potential protein targets for sinomenine and morphine addiction

PharmMapper (21) and SwissTargetPrediction (22) were used to predict the potential protein targets for sinomenine. The information with regards to the chemical structure of sinomenine was obtained from PubChem database (23) where the SDF file was then used for PharmMapper server and canonical SMILES string for SwissTarget Prediction. Only the protein targets having more than zero probability value in SwissTargetPrediction and more than 0.7 normal fit value in PharmMapper were chosen. g:Profiler web server was utilize to convert the results from PharMapper to gene name (24). Those protein targets were combined and any duplicates were removed. The potential protein targets for morphine addiction were obtained by DisGeNet (25) and GeneCards (26) databases. The keywords used for the search are “Morphine Dependence” and “Morphine Addiction”. Protein targets having disease gene association score less than 0.2 in DisGeNet and relevance score more than 1 in GeneCards datasets were chosen for analysis. The targets were combined and any duplicate targets were removed. Overlapping protein targets of sinomenine and morphine addiction were identified using a Venn diagram drawn in Venny (27).

Protein-protein interactions (PPI) and Network Analysis
The protein-protein interactions of the overlapping targets were analysed using GeneMANIA online tool (28). Homo sapiens was chosen as the type of organism and default settings were chosen. PPI network obtained from GeneMANIA was uploaded to Cytoscape (29) for network analysis. Topological network analysis was conducted to identify the most important nodes (genes) using degree centrality (DC), betweenness centrality (BC), and closeness centrality (CC) criteria.

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway analysis

The list of overlapping protein targets was submitted to ShinyGO 0.77 online tool (30) for GO and KEGG pathway analysis. Homo sapiens was chosen as the type of organism and default settings were chosen. The FDR cutoff value was set to 0.05 and the number of pathways was limited to 10 to obtain only the pathways highly associated with the interactions between sinomenine and morphine addiction.

Molecular docking

The crystal structures of the protein targets were retrieved from Protein Data Bank (PDB) database (31). The crystal structures of the protein targets were prepared using AutoDock Tool (32) where the water molecules were removed and the polar hydrogen and

Kollman charges were added. Open Babel was used to convert sinomenine chemical structure format from SDF to PDB format. Prior to docking, the structure was minimized using MM2 forcefield. Molecular docking was conducted using AutoDock Vina (33) following the identification of the binding site coordinates. Binding conformations with the lowest binding energy were considered to be the most stable. Finally, the protein-ligand binding interactions were visualized using Discovery Studio. Overall, the methodology used in this study was illustrated in Fig. 1A..

RESULTS

Protein targets for sinomenine associated to morphine addiction

A total of 346 (100 + 246) protein targets for sinomenine were identified using SwissTargetPrediction and PharmMapper web server, respectively. After the removal of the duplicates and protein targets with zero probability value in SwissTargetPrediction and normal fit value less than 0.7 in PharmMapper, the remaining of 78 targets were used for further analysis. For morphine addiction targets, a total of 414 (44 + 370) protein targets were identified using DisGeNet and GeneCards databases. After the removal of the duplicates, protein targets with disease gene association score less than 0.2 in DisGeNet and protein targets with less than 1 for relevance score in GeneCards, a remaining of 301 targets associated with morphine addiction were obtained.

A total of 15 protein targets were observed to be overlapped between sinomenine predicted targets and morphine addiction targets, visualized using a Venn diagram (Fig. 1B). Those protein targets include OPRM1, OPRD1, HTR3A, NOS1, CSNK2A1, MAOB, MMP9, MAPK8, BCHE, HSP90AA1, SRC, AR, CES1, OPRK1 and HRH1 (Table I).

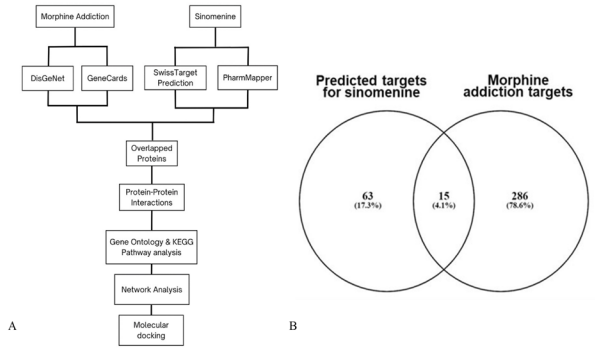

Fig. 1: Flowchart showing the methodology used in the study (A) and Venn diagram showing overlapping protein targets (B).

Table I: Protein targets for sinomenine associated to morphine addiction.

No.	Protein targets	Name
1	OPRM1	Mu opioid receptor
2	OPRD1	Delta opioid receptor
3	HTR3A	Serotonin 3a (5-HT3a) receptor
4	NOS1	Nitric-oxide synthase, brain
5	CSNK2A1	Casein kinase II alpha
6	MAOB	Monoamine oxidase B
7	MMP9	Matrix metalloproteinase 9
8	MAPK8	c-Jun N-terminal kinase 1
9	BCHE	Cholinesterase
10	HSP90AA1	heat shock protein 90 alpha
11	SRC	Proto-oncogene tyrosine-protein kinase Src
12	AR	Androgen receptor
13	CES1	Liver carboxylesterase 1
14	OPRK1	Kappa Opioid receptor
15	HRH1	Histamine H1 receptor

Gene Ontology and KEGG pathway analysis

Those 15 protein targets were subjected to GO and KEGG pathway enrichment analyses. The top 10 biological processes (BP), molecular functions (MF), cellular components (CC) and KEGG pathways are visualized in Fig. 2. Fold enrichment in the chart shows how considerably the genes of a certain pathway are overrepresented. The chart presented illustrated that BP was significantly enriched with (i) G protein-coupled opioid receptor signalling pathway, (ii) peptidyl-serine phosphorylation, (iii) peptidyl-serine modification, (iv) response to lipopolysaccharide, (v) response to molecule of bacterial origin, (vi) response to lipid, (vii) response to organic cyclic compound, (viii) response to abiotic stimulus, (ix) response to oxygen-containing compound and (x) cell-cell signalling.

The main GO terms for MF were related to (i) G protein-coupled opioid receptor activity, (ii) neuropeptide binding, (iii) receptor serine/threonine kinase binding (iv) scaffold protein binding, (v) peptide receptor activity, (vi) peptide binding, (vii) amide binding, (viii) signalling receptor activity and (ix) molecular transducer activity cholinesterase activity. CC were enriched in (i) integral component of presynaptic membrane, (ii) intrinsic component of postsynaptic membrane, (iii) dendrite, (iv) dendritic tree, (v) somatodendritic compartment, (vi) axon, (vii) neuron projection and (viii) synapse. The essential signalling pathways of sinomenine in morphine addiction were presented by KEGG pathway enrichment analysis. The top 10 pathways primarily focus on mitophagy, IL-17 signalling pathway, endocrine resistance, relaxin signalling pathway, prostate cancer, inflammatory mediator regulation of

TRP channels, estrogen signalling pathway, fluid sheer stress and atherosclerosis, lipid and atherosclerosis and neuroactive ligand-receptor interaction.

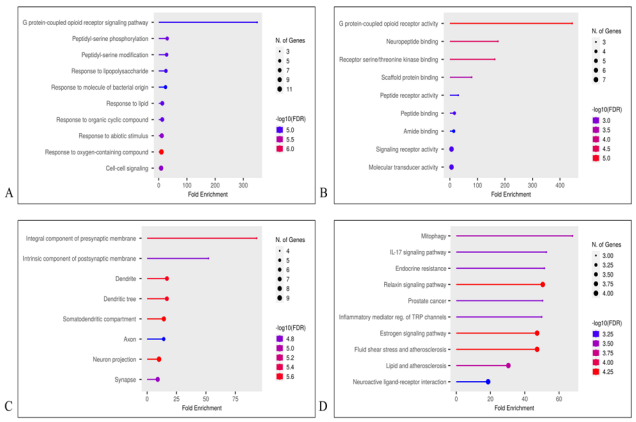

Fig. 2: GO and KEGG enrichment analysis of sinomenine-morphine addiction overlapping genes. The top 10 terms of (A) BP, (B) MF, and (C) CC obtained by GO enrichment analysis. (D) KEGG pathway enrichment analysis results in the top 10 pathways. Abbreviations: BP, biological process; MF, molecular function; CC, cell composition.

Protein-protein interactions (PPI)

A PPI analysis was conducted using the GeneMANIA database to elucidate the interactions of these overlapping protein targets (Fig. 3A). The results of GeneMANIA also revealed that the functions of these protein targets interacted with each other and the number of interactions of each gene, which was found that 37.94% shared protein domains, 19.64% physical interactions 14.69% predicted, 12.58% had co-expression, 8.81% pathway, 5.83% co-localization and 0.50% genetic interactions.

Topological Network Analysis

The results for the topological network analysis were shown in Table II. The protein target that has the highest degree centrality value is OPRD1 followed by HSP90AA1, OPRK1, CSNK2A1, SRC, AR, OPRM1, NOS1, MAPK8, HTR3A, BCHE, MAOB, MMP9, HRH1 and CES1.

Table II: The topological analysis of sinomenine-morphine addiction network.

Target name	Abbreviation	Degree	Closeness	Betweeness
Delta opioid receptor	OPRD1	12	0.608695652	0.260714286
heat shock protein 90 alpha	HSP90AA1	11	0.608695652	0.162467295
Kappa Opioid receptor	OPRK1	9	0.538461538	0.058974359
Casein kinase II alpha	CSNK2A1	9	0.538461538	0.053676086
Proto-oncogene tyrosine-protein kinase Src	SRC	7	0.56	0.128074307
Androgen receptor	AR	7	0.538461538	0.240750916
Mu opioid receptor	OPRM1	6	0.451612903	0.015044479
Nitric-oxide synthase, brain	NOS1	4	0.4375	0.022370487
c-Jun N-terminal kinase 1	MAPK8	4	0.5	0.047488226
Serotonin 3a (5-HT3a) receptor	HTR3A	3	0.5	0.133241758
Cholinesterase	BCHE	3	0.411764706	0.054945055
Monoamine oxidase B	MAOB	3	0.424242424	0.017765568
Matrix metalloproteinase 9	MMP9	2	0.4375	0.019230769
Histamine H1 receptor	HRH1	2	0.4	0.038003663
Liver carboxylesterase 1	CES1	2	0.388888889	0

Molecular Docking

To elucidate the binding energy and binding conformation of the sinomenine within the potential protein targets, molecular docking was done where the results were tabulated in Table III and visualized in Fig. 4. Lower binding energy (more negative) represent higher affinity for sinomenine to bind to the specific protein targets. It was observed that the protein target with the lowest binding energy is CES1 (-8.8 kcal/mol) followed by BCHE (-8.7 kcal/mol), OPRM1 (-8.4 kcal/mol), NOS1 (-7.9 kcal/mol), OPRD1 (-7.3 kcal/mol), SRC (-7.2 kcal/mol), OPRK1 (-6.9 kcal/mol), HTR3A (-6.8 kcal/mol), HSP90AA1 (-6.7 kcal/mol), MAOB (-6.1 kcal/mol), MMP9 (-6.1 kcal/mol), CSNK2A1 (-5.9 kcal/mol), MAPK8 (-5.6 kcal/mol), HRH1 (-5.1 kcal/mol) and AR (-4.1 kcal/mol).

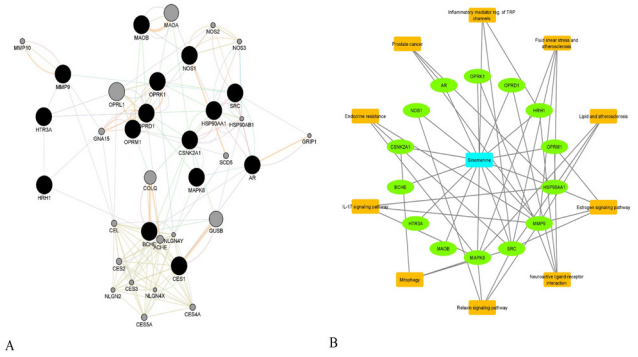

Fig. 3: PPI network of 15 overlapping targets (A) and sinomenine-target-pathway network (B). Black and green nodes both refer to the target proteins.

Table III: Molecular docking analysis result of sinomenine with 15 potential protein targets.

Protein targets	PDB ID	Binding energy (kcal/mol)	Number of hydrogen bonds	Hydrogen bond interaction residue	Other interaction residue
CES1	1YA4	-8.8	1	Conventional hydrogen bond: His468	Alkyl: Leu97, Val146 and Leu363
BCHE	3O9M	-8.7	5	Conventional hydrogen bond: Gly117, Ser198 and His 438 Carbon hydrogen bond: His438 and Gly439	Pi-alkyl: Phe329 and His438
OPRM1	5C1M	-8.4	3	Carbon hydrogen bond: His54, Asp147 and Lys233	Pi-alkyl: Trp318 Pi-sigma: Val300
NOS1	4UCH	-7.9	7	Conventional hydrogen bond: Ser418 and Arg419 Carbon hydrogen bond: Asn416, Leu705 and Phe709 Pi-donor hydrogen bond: Arg704	Pi-alkyl: Arg704
OPRD1	4N6H	-7.3	1	Carbon hydrogen bond: Lys214	Pi-alkyl: Trp284 Alkyl: Val281 Pi-sigma: His278 and Val281
SRC	3EL8	-7.2	2	Carbon hydrogen bond: Met341 and Ala390	Pi-alkyl: Leu393 and Ala403 Alkyl: Leu393 Pi-pi T-shaped: Phe405
OPRK1	6B73	-6.9	2	Conventional hydrogen bond: Cys210 Carbon hydrogen bond: Asn122	Pi-alkyl: Trp124 Alkyl: Val118 and Cys210 Pi-sigma: Val118
HTR3A	6W1J	-6.8	3	Conventional hydrogen bond: Tyr207 Carbon hydrogen bond: Trp63 and Asp202	Pi-alkyl: Trp156 Pi-sigma: Trp156
HSP90AA1	5H22	-6.7	1	Carbon hydrogen bond: Asn51	Pi-alkyl: Phe138 Alkyl: Leu48 and Val186
MAOB	2Z5X	-6.1	4	Carbon hydrogen bond: Tyr69, Ile180, Gln215 and Lys305	Pi-alkyl: Tyr407 Pi-pi stacked: Tyr407 and Tyr444
MMP9	1GKC	-6.1	3	Conventional hydrogen bond: His405 and His411 Carbon hydrogen bond: Glu402	Pi-alkyl: His411 Pi-pi T-shaped: His411
CSNK2A1	3OWJ	-5.9	3	Conventional hydrogen bond: His160 Carbon hydrogen bond: Asp120 and His160	Pi-alkyl: Leu45 and His160 Alkyl: Val53
MAPK8	2XRW	-5.6	1	Conventional hydrogen bond: Arg192	Pi-alkyl: Ala36 Pi-cation: Lys153
HRH1	3RZE	-5.1	2	Carbon hydrogen bond: Asp107	Pi-alkyl: Tyr108, Trp158 and Phe435 Pi-sigma: Trp428 Pi-pi stacked: Phe432 Pi-pi T-shaped: Tyr108 and Phe435
AR	1E3G	-4.0	1	Carbon hydrogen bond: Met742	Pi-alkyl: Leu704 Pi-sigma: Met745

Construction of drug–target–disease network

To further explore the molecular mechanism of sinomenine for morphine addiction treatment, we constructed a "sinomenine–target–pathway" network (Fig. 3B). The green nodes represent the target genes, the blue node represent sinomenine, the orange nodes represent the pathways involved, and the edges represent interactions. The construction of the "sinomenine–target–pathway" network can show the relevant targets of sinomenine for morphine addiction treatment more visually.

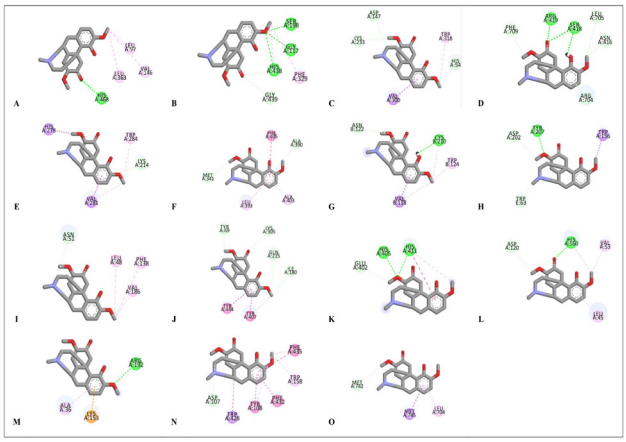

Fig. 4: Visualization of binding interactions between sinomenine and the protein targets: CES1 (A), BCHE (B), OPRM1 (C), NOS1 (D), OPRD1 (E), SRC (F), OPRK1 (G), HTR3A (H), HSP90AA1 (I), MAOB (J), MMP9 (K), CSNK2A1 (L), MAPK8 (M), HRH1 (N) and AR (O).

Based on the visualization of the binding interaction between sinomenine and the protein targets, it can be seen that sinomenine can form both hydrogen and hydrophobic interactions within the binding site of the predicted protein targets. Sinomenine forms the highest number of hydrogen bond interactions which is seven upon interacted with NOS1 and this includes three conventional hydrogen bonds, three carbon-hydrogen bonds and one pi-donor hydrogen bond while the least hydrogen bond interaction was observed when sinomenine interacted with CES1, OPRD1, HSP90AA1, MAPK8 and HRH1 where only one hydrogen bond interaction was observed either conventional hydrogen bond or carbon-hydrogen bond. Other than hydrogen bond interaction, sinomenine also can form other types of interactions such as pi-alkyl, alkyl, pi-sigma, pi-pi T-shaped, pi-cation and pi-pi stacked interactions with the protein targets. Excluding CES1, sinomenine can form one to three pi-alkyl interactions with almost all of the protein targets.

DISCUSSION

Previous studies have reported on the promising potential of sinomenine to be explored further as pharmacological agent to treat morphine addiction (10). It was reported that sinomenine itself did not cause physical dependence rendering it as a safe agent and less likely to be abused (14). Sinomenine also is capable in attenuating morphine-induced place preference in animal study using mice and also zebrafish (11-13). Employing a network pharmacology approach, our analysis identified 15 potential protein targets related to morphine addiction where sinomenine can interact. These protein targets include OPRM1, OPRD1, HTR3A, NOS1, CSNK2A1, MAOB, MMP9, MAPK8, BCHE, HSP90AA1, SRC, AR, CES1, OPRK1 and HRH1. Network topology and molecular docking analysis were subsequently done which demonstrated the degree centrality values of the protein targets ranging from two until 12 while the binding energy of sinomenine at the protein targets ranging from -4.0 kcal/mol to -8.7 kcal/mol.

Firstly, it can be observed that opioid receptors namely delta opioid receptor (OPRD1), kappa opioid receptor (OPRK1) and mu opioid receptor (OPRM1) are some of the main targets for sinomenine in the modulation of opioid addiction pathway. Not only that, GO analysis also revealed that G protein-coupled opioid receptor signalling pathway and G protein-coupled opioid receptor activity are the most enriched with regards to the sinomenine's mechanisms in modulating drug addiction. Molecular docking analysis indicated that sinomenine could bind and interact with OPRD1, OPRK1 and OPRM1 with binding energy values of -7.3 kcal/mol, -8.4 kcal/mol and -6.9 kcal/mol respectively. Network topology analysis also showed degree centrality values of 12 for OPRD1, nine for OPRK1 and six for OPRM1. The binding energies of less than -6.0 kcal/mol and degree centrality values of six and above indicated that the interactions of sinomenine and the opioid receptor systems are the main mechanism where sinomenine could exerts its effect as potential anti-addiction agent.

Previous studies have reported the involvement of opioid receptors in the effects of sinomenine especially OPRD1 and OPRM1. Sinomenine at dose of 80 mg/kg was able to upregulate OPRD1 and OPRM1 receptor expression on the zebrafish brains (34). In another study, the same dose was applied to the mice and the OPRM1 receptor expression was increased in the mice brain but not OPRD1 receptor expression (12). In another study focusing on the antinociceptive effects of sinomenine, antinociception induced by sinomenine was attenuated when opioid receptor antagonist (naloxone) and selective mu opioid receptor antagonist (β-funaltrexamine) were given but not delta opioid receptor antagonist (naltrindole) and kappa opioid receptor antagonist (nor-binaltorphimine) (35).

Since morphine itself binds and activates opioid receptors particularly OPRM1 to produce euphoria which eventually can lead to opioid addiction, it is very much possible for sinomenine to interact with the opioid receptors thus preventing the binding of morphine, its rewarding effect and also the development

of morphine addiction. Not only that, the interactions of sinomenine at opioid receptors could treat the addiction by modulating the activity of opioid receptors and also the activation reward pathway. For example, currently approved medications to manage opioid addiction rely on the drugs' action at the opioid receptors such as methadone (OPRM1 agonist), naltrexone (OPRM1 antagonist) and buprenorphine (OPRM1 partial agonist and OPRK1 antagonist) indicating that the drugs targeting opioid receptors can be a promising treatment to treat opioid addiction provided they come with a good safety profile and devoid of opioid receptor-associated side effects (36).

Other than the opioid receptors, predicted protein targets that have been found to interact with sinomenine to a lesser extent include SRC, NOS1, BCHE, CES1, HTR3A, HSP90AA1, MAOB, MMP9, MAPK8, HRH1, CSNK2A1 and AR. Some of the interactions of these protein targets with sinomenine and morphine addiction have been described previously. Sinomenine was reported to be able to suppress the SRC activation due to its anti-inflammatory activity (15). Suppression or inhibition of SRC activity was associated with attenuation of morphine-induced tolerance and might be a good strategy to treat drug addiction since SRC is involved in the regulation of Tau protein which is important in the drug-associated memory formation (37, 38). NOS1 expression and activity on the other hand, could be downregulated by sinomenine in modulating the effects of morphine (14). It has been reported that the inhibition of protein–protein interactions involving NOS1 decreased morphine reward and relapse like behaviour particularly by the reduction in the production of nitric oxide where its excessive production has been linked to opioid dependence (39). With regards to HRH1, sinomenine might bind and activate HRH1 since sinomenine promotes histamine release and activation of HRH1 has been described as the promising treatments to treat opioid use disorder (40, 41).

For the rest of the protein targets, there are very little information on their relationship with sinomenine and morphine addiction. Nevertheless, some protein targets have been linked to the development of morphine addiction such as BCHE where its inhibitor was able to weaken reduce morphine-induced place preference in animal study (42), MAOB level that increased in the nucleus accumbens of rat offsprings following parental exposures towards morphine (43), HTR3A where its antagonist ondansetron inhibited the developments of non-chronic opioid use disorder (44), MAPK8 involvement in learning and memory (45), HSP90AA1 inhibition which blocked antinociceptive effects of morphine (46), MMP9 contribution to morphine dependence (47) and AR modulation of pharmacological effects of opioid (48). Considering the degree centrality values for all protein targets in network topology analysis coupled with the binding energy values from molecular docking, it was proposed that OPRD1, OPRM1, SRC, OPRK1 and NOS1 are the top five most significant targets for sinomenine in treating morphine addiction.

GO analysis revealed that neuropeptide binding, receptor serine/threonine kinase binding, integral component of presynaptic membrane and intrinsic component of postsynaptic membrane were enriched compared to the others while KEGG pathway analysis showed that mitophagy as the most enriched pathway followed by interleukin-17 (IL-17) signalling pathway, endocrine resistance, relaxin signalling pathway, prostate cancer, inflammatory mediator regulation of TRP channels, estrogen signalling pathway and fluid shear stress and atherosclerosis. Opioid uses and exposures were associated with the alteration in mitochondrial function in both brain and body which can impact the neuronal signalling and addiction behaviour (49). The involvement of the mitophagy pathway suggests that sinomenine may play a role in the regulation of damaged or dysfunctional mitochondria. Previously, it was described that sinomenine possess mitochondrial protective properties by reducing oxidative stress and inhibiting the mitochondrial apoptotic pathway via the inhibition of Bax translocation and Cyt c release from the mitochondria (50).

Nevertheless, there is a limitation in our current study. It is important to note that the proposed pharmacological mechanism for sinomenine in treating morphine addiction relied on the computational approaches only thus necessitates additional confirmation through further experimental validations and clinical investigations.

CONCLUSION

In summary, this study has identified 15 morphine addiction-related targets that might interact with sinomenine, unravelling the molecular mechanisms involved in sinomenine effects on morphine addiction. However, further experimental studies are necessary to validate these interactions and explore the functional consequences. GO analysis revealed insights into the BP, MF, and CC associated with sinomenine effects, providing clues about its role in neurotransmission, stress response, and other addiction-related pathways. The KEGG pathway analysis highlighted important signalling pathways, such as mitophagy, IL-17 signalling, and endocrine resistance, suggesting potential mechanisms through which sinomenine exerts its effects in morphine addiction. Overall, these findings will advance our understanding of sinomenine's therapeutic potential in morphine addiction and provide a foundation for future research to elucidate its specific mechanisms of action. Further exploration and validation of these interactions will contribute to a more comprehensive understanding of sinomenine's effects and its potential as a treatment option for morphine addiction. Future studies focusing on the pharmacological effects of sinomenine in

other types of addiction such as methamphetamine addiction are recommended looking at the increase in methamphetamine cases reported.

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